

Nutritional Strategies of Animal Feed Additives

Abdel Fattah Z. M. Salem
Editor

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NUTRITIONAL STRATEGIES OF ANIMAL FEED ADDITIVES

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ABDEL FATTAH Z. M. SALEM
EDITOR

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PREFACE

Quality of feed nutrition is influenced not only by the content but also by some other aspects such as, feed presentation, hygiene, anti-nutritional factors, digestibility and palatability. Feed additives provide a mechanism by which such dietary deficiencies can be addressed which benefits not only the nutrition and thus the growth rate of the animal concerned, but also its health and welfare. Feed Additives could to alter animal production, ruminal metabolism, digestion that is not considered itself a nutrient. A lot of feed additives contain sodium in sodium bicarbonate or protein in yeast culture. Some common feed additives contain sodium bicarbonate, magnesium oxide, yeast culture, direct fed microbials, niacin, zinc-methionine, ionophores, biotin, antibiotics, and exogenous enzyme preparations. Feed additives could be modify animal metabolism in specific and directed ways by improving productive efficiency (weight gain or milk yield) improving carcass composition in growing animals, increasing milk yield in lactating animals and decreasing animal waste per production unit. Feed additives could be divided into five groups; a)-Technological additives which influences the technological aspects of the feed to improve its handling or hygiene characteristics. b)- Sensory additives which improve the palatability of a diet by stimulating appetite, usually through the effect these products have on the flavour or colour of the diet. c)- Nutritional additives, such additives are specific nutrient(s) required by the animal for optimal growth. d)- Zootechnical additives which improve the nutrient status of the animal, not by providing specific nutrients, but by enabling more efficient use of the nutrients present in the diet. e)- Coccidiostats and Histomonostats which used to control intestinal health of poultry through direct effects on the parasitic organism concerned.

Our present text book has 14 chapters discussed the impacts of some of these feed additives on animal metabolism, health and production. Two chapters were discussed the impact of exogenous enzymes on animal nutrition and metabolism (chapters 1 and 5), while chapters 7 and 8) were discussed the impact of Zilpaterol in ruminant feeding (chapter 8) and carcass characteristics (chapter 7). Impacts of selenium on immunity system of animals and carcass characteristics and meat quality were also discussed in chapters 9 and 10. Antibiotics in animal nutrition were outlined in chapter 2 of the present book. Some useful information about the impact of yeast on horse nutrition is presented in chapter 6.

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Chapter 1

ANAEROBIC ENZYMES AS A NEW TECHNOLOGY IN ANIMAL FEED

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ABSTRACT

Biological treatment is a new method for the improving the nutritive value of lignocelluloses materials which are the most abundant in agricultural residues. The most common cellulolytic microorganisms are fungal cultures (*Aspergillus oryzae* and *Saccharomyces cerevisiae*) and actinomycetes and other bacteria which have the ability to produce sufficient amount of cellulase and hemi-cellulase enzymes which are capable of degrading the lignocelluloses material [1]. The biological treatments include bacterial, fungal and enzymatic additives. Each microbial species possesses a unique combination of characteristics, including substrates utilized, types and ratios of fermentation products, and growth yield [2]. The attachment of microbes to feed particles and the nitrogenous substances play important roles in the biosynthesis of protein by different organisms [3]. Thus, in order to increase digestibility of these roughages, it is important to destroy the linkage between cellulose, hemi-cellulose and lignin or to destroy the compact nature of the tissue, so that lignified tissue is separated from non-lignified tissues. There have been attempts to do that by mechanical, chemical or biological treatments and enriching treatments. Whereas, physical treatment means that reducing particle size for ensiling and install-feeding and that reduction of particle size can be achieved using a power driven chopper or a hand operated chaff cutter; this mean that the surface area of non-lignin material exposed to microbial attack in the rumen is increased, thus increasing the rate of digestion, thereby reducing a possible limitation to intake [4] and the smaller the particle size the less scope there is for selection.

The use of feed enzymes in feed ruminants has been viewed with considerable skepticism, but in recent years a considerable number of studies on this topic have been

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conducted. The majority of these experiments were designed with the expectation that a fibrolytic enzyme should increase the degradability of feed in the rumen, and this response has been observed in many of these studies [5, 6]. However, lack of effects [7, 8] or even negative effects [9] have also been detected. These inconsistencies could be attributed to differences in crude enzyme preparations, type of diets fed to the animals and enzymes application methods [10]. Exogenous fibrolityc enzymes might enhance attachment and/or improve access to the cell wall matrix by ruminal microorganisms and by doing so, accelerate the rate of digestion [11]. Due to the importance of forage in ruminant feeding, the possibility of improving its nutritive value by adding exogenous enzymes should be explored. Most of studies with enzyme-treated forages have been conducted with good-quality forages, but limited data are available involving application of exogenous enzymes to fibrous feeds.

In this chapter, we aim to help solve this problem with nutritional scientists and their efforts to find methods to improve the utility of these by-products in feeding ruminants. Enzymes have been recently used in the ruminant rations to degrade fiber contents to make it easier for digestion, to be more useful for the micro-organisms and flora which are the main source of the microbial protein in the ruminal media and to reduce the costs of the ration.

WHAT ARE ZAD® AND ZADO®?

The enzyme product of ZAD® is a bio-technical product made from natural sources to elevate the level of cellulase enzymes from anaerobic bacteria which can convert the polysaccharide into monosaccharide by specific enzymes, such as cellulase 8.2 U/g and hemicellulase 6.2 U/g, in addition to activation of amylase 64.4 U/mg and protease 12.3 U/g. The two products are intended to provide tools to improve the nutritive value for fibrous materials through ZAD® and ZADO® is designed to improve the rumen kinetics and overall animal digestion.

The ZAD® is used in treating a lot of agricultural byproducts such as rice straw, corn stalks, bagasse, etc… Also it was applied to (dried distillers grains with solubles) and citrus pulp. This mix between anaerobic enzymes and those by-products ended up making improved products from the nutritive point of view, which provides a new line of animal feed that will help in reducing the feed cost. It will also create new products to help give nutritionists the freedom of choice in building up more suitable feeding programs.

The anaerobic enzyme matrix of ZAD® and ZADO® helps in breaking down the secondary compounds in the feed. It also increases the microbial populations, which will lead to an increase in the microbial protein either in the reticulo-rumen area or by-pass through the digestive tract. An increase in the total volatile fatty acids is noticed. In some research work an improvement in the animal's immunity is observed. An improvement in the total digestable nutrients of the ration is recorded in all trials. That goes hand by hand with the elevations of the animal digestion coefficients in large or small ruminants.

IN DAIRY ANIMALS

Positive effects of adding exogenous enzymes to ruminant diets have been reported for lactating dairy cows and growing cattle. Dairy cows fed forage treated with a fibrolytic

enzyme additive ate more feed and produced 5–25% more milk, improved the energy balance of transition dairy cows and increased milk production in small ruminants.

In feedlot cattle, fibrolytic enzymes have improved live weight gain by as much as 35% and feed conversion ratio by up to 10%. A commercial exogenous enzyme mixture (ZADO®), prepared from anaerobic bacterium, has been shown to improve ruminal fermentation, N balance and nutrient digestibility, as well as milk yield of cows fed diets containing Egyptian by-product feeds as well as LW gain and feed conversion of wheat straw in sheep and goats [12, 13, 14, 15].

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Output of milk and milk energy was higher for cows fed the enzyme supplemented diet However, increased milk yield for cows fed with enzymes was not accompanied by increases in yield of milk components, except crude protein (CP), which was increased by the addition of enzyme products [16]. In addition some studies of the effect of ZADO® on dairy cows performance is listed in table 1.

NUTRIENT INTAKE AND DIGESTIBILITY

Rode et al. [21] found that the digestibility of total mixed rations contained 24% corn silage, 15% alfalfa hay, and 61% barley concentrate was higher for cows fed the enzyme supplement, except for starch digestibility, for which enzyme supplementation had only a minor effect. The digestibility (%) were dry matter (DM) 61.7, 69.1; organic matter (OM) 63.9, 70.6; neutral detergent fiber (NDF) 42.5, 51.0; acid detergent fibre (ADF) 31.7, 41.9; Starch 94.1, 95.5; CP 61.7, 69.8. They found that Intake of DM and other nutrients was not affected by enzyme supplementation and this lack of effect of enzymes on dry matter intake (DMI) was consistent throughout the experiment.

Fifty Holstein cows in an experiment were used to evaluate the dose response to a direct-fed cellulase and xylanase enzyme mixture applied to the forage portion (60% corn silage and 40% alfalfa hay) of a total mixed ration just prior to feeding. Cows were fed one of five treatment diets for 12 weeks.

Diets 1 through 4 were 55% forage: 45% concentrate and consisted of an untreated control and the control diet plus 0.7, 1.0, or 1.5 L of enzyme concentrate/ton of forage DM. They found that DMI were similar for cows fed all diets, and all diets were readily consumed [22].

Table 1. Doses and effects of ZADO® on dairy cows performance

Doses (g/h/d)	Animal	Effects	Ref.
20	Lactating Baladi Goats	Increasing the milk production by 5.5%	[17]
40	Brown Swiss cows	Increased milk production for 12.8 to 15.7 L/head/day	[16]
40	Friesian cows	Increased milk production for 24.2 to 26.1 L/head/day	[18]
40	Brown Swiss cows	Increased milk production by 20% / head /day	[19]
20	Lactating ewes	Increased milk production by 26% / head /day	[20]
40	Friesian cows	Increased milk production by 25% under heat stress	[15]

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Digestion of the NDF fraction by lactating cows was numerically greater after 12h of incubation for forages treated with a low level of a cellulase and xylanase enzyme and high levels of a cellulase and xylanase enzyme mix, respectively, compared to untreated forage, but these differences were not statistically different. After 48 h, NDF digestion of forage treated with a high level of a cellulase and xylanase enzyme mix was lower than the control but not different from forage treated with low levels of an alternative cellulose and xylanase enzyme mix. The diets had similar chemical compositions. The DMI was not affected by treatment and averaged 22.1 kg/d [23].

Digestibility of OM, NDF and ADF was increased in comparison to the control when enzymes were added to the entire concentrate on the ration of lactating Holstein cows. They found that cows receiving the enzyme applied to concentrate had a significantly higher DM and OM digestion compared to cows receiving the control or the enzyme applied to premix. Digestion of NDF and ADF digestion was also increased in enzyme products added to concentrate fed cows compared to control. Cows receiving enzyme products added to supplement and enzyme products added to premix treatments showed a non-significant, intermediate increase in nutrient digestibility compared with control. The digestibility (%) were: 65.1,72.6,70.1 and 65.9 for DM; 66.6, 73.8, 71.4 and 67.2 for OM; 44.3, 55.6, 50.6 and 46.9 for NDF; 43.6, 55.6, 50.2 and 47.4 for ADF for treatments control, enzyme applied to concentrate, enzyme applied to supplement, enzyme applied to premix. They found that DM and other nutrient intakes were not significantly altered by inclusion of the enzyme product [24]. Holstein dairy cows were fed to evaluate production responses to cellulase, xylanase and ferulic acid esterase enzyme application on the forage portion of the diet. They found that cows fed diets containing untreated forage or enzyme-treated forage had similar DMI [25].

Digestibility of DM was similar for control and enzyme-supplemented diets in early lactation cows, but in late lactation cows, DM digestibility was numerically greater with the enzyme addition compared to the control. The values were 64.0, 63.9, 58.2 and 61.1 for control and enzyme for early and late lactation respectively.

Apparent NDF digestibilities were not affected by enzyme treatments. Apparent P digestibility, milk phosphorus (P), and P retained in body tissue were not significantly affected by enzyme treatment or by the interaction of stage of lactation and treatment. They found that DMI was numerically, but not significantly, greater in cows fed the diet containing the enzyme formulation compared to those fed control diets. The values were DMI, kg/d 25.5, 26.7, 24.3 and 24.3 for control and enzyme, for early and late lactation respectively [26].

Biological treatments on chemical composition and *in vitro* and *in vivo* digestibilities of poor quality roughages; they found that there were differences among treatments detected in DM, OM, crude fibre (CF), nitrogen free extract (NFE), ADF, hemicellulose and cellulose digestibilities.

Also, the differences between treatments in CP, ether extract (EE) and NDF digestibilities were significant. All biological treatments increased the values of nutrient digestibility coefficients than those in control. Rations treated with fungal treatments showed the highest digestion coefficient for all nutrients followed by yeast treatment and then control, which recorded the lowest values of nutrient digestibilities [27].

The values of DMI were 20.67, 21.13, 20.86 and 20.43 for control; enzyme sprayed on total mixted ration (TMR); enzyme sprayed on concentrates; enzyme infused into rumen. Total tract DM digestibility did not differ significantly from control for any of the treatments but was significantly higher on enzyme sprayed on TMR than on enzyme sprayed on

concentrates. There were no differences in intake of NDF, NDF digestibility in the rumen was lower on the enzyme sprayed on TMR than on control or enzyme product infused into rumen [28].

Supplementing diets of Awassi ewes and Shami goats with exogenous fibrolytic enzyme on birth and weaning weight of lambs and kids and on milk production of dams in the first 60 days of lactation had no effect on feed intake. The DMI for treated and untreated diet with enzymes was (kg per head per day) 2.28, 2.30, 2.19 and 2.23 for Awassi ewes and Shami goats.

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They studied the efficacy of direct feeding a fibrolytic enzyme on lamb digestibility and on the performance of growing milk-type calves and kids. They found that fibrolytic enzymes increased DM and OM digestibilities of treated lambs compared to those of control. A similar trend was observed for the CF, NDF and ADF digestibility coefficients. Treated lambs had higher digestibilities than untreated ones. There were no differences observed in CP digestibility between treated and control lambs [29].

Treated corn straw and bagasse with ZAD® compound, they found that ZAD® treatments increased digestibility of DM, OM, CP, EE, NFE, NDF, hemicelluloses and Cellulose compare with untreated material [30]. Degradabilities of DM, NDF, and hemicellulose from ammonia untreated rice straw at 24 h of incubation were not affected by cellulases or xylanases enzymes.

There was, however, an increase in ADF degradation using cellulases and degradability of the nonfibrous fraction was increased using xylanases. Degradability of the fibrous fractions (NDF, ADF, and hemicellulose) increased with proteases, while a combination of endoglucanases, xylanases, and a fibrolytic enzyme, increased DM degradability mainly due to increased degradability of the non-fibrous fraction [31]. The properties used to improve the nutritional value of poor quality roughages (berseem hay, bagasse, wheat straw and rice straw) were biological treatments such as fungi (*Trichoderma reesei* and *Trichoderma kongei*) and fibrolytic enzymes mixture (1, 2 and 3%). He found that DMI was slightly increased with animals fed on treated silage compared with control [32].

The effects of an *Aspergillus oryzae* extract containing amylase activity on performance and carcass characteristics of finishing beef cattle. Supplementation of α-amylase increased DMI and average daily gain (ADG). The increases in ADG were related to increased DMI and efficiency of gain during the initial 28-d period but were primarily related to increased DMI as the feeding period progressed rates in the low to medium range [33].

The nutritive value of *Atriplex halimus* (AH) foliages in presence of ZADO® in sheep was also evaluated. Addition of ZADO® to either fresh AH or sun-dried AH diets improved all nutrient compounds digestibility. Total feed intake was unaffected by sun-dried or ZADO® treatments. Generally, addition of ZADO® to AH-S diet had the highest improvement of feed intake, digestibility and nitrogen utilization than the other treatments [13].

The effect of ZADO® on feed intake, apparent digestibility and animal growth performance of sheep and goats fed wheat straw *ad lib*. as a basal diet and commercial concentrate with or without 10g/animal/d of ZADO®; feed intake was not affected by the addition of ZADO® in sheep or goats but it improved the nutrients digestibility coefficients as well as the total digestible nutrient of feed in sheep and goats. Addition of ZADO® increased the NDF digestibility of diet and the improvement was more in goats than sheep [34].

RUMINAL FERMENTATION AND MICROBIAL PROTEIN SYNTHESIS

Ruminal pH, was lower for steers receiving enzyme treatments than for control (water applied to grass forage at feeding) (5.97 vs. 6.03). The values were 6.03, 6.01, 5.97, 5.92 and 5.96 for treatments control, enzyme applied to grass forage 24 h before feeding, enzyme applied to grass forage at feeding enzyme applied to barley supplement at feeding, enzyme ruminally infused 2 h after feeding. Ruminal control, concentrations of volatile fattey acids (VFA) was greater at 16 h for steers fed enzyme treatments (average 136.5 mm/L) than for control, (104.0 mm/L). Molar percentage of individual ruminal VFA was not affected by treatment. Steers fed enzyme treatments tended to have greater ruminal NH_3-N than control, whereas it was lower in steers receiving the enzyme applied to the barley supplement at feeding treatment than enzyme applied to grass forage 24 h before feeding and enzyme applied to grass forage at feeding. The values were 4.5, 5.5, 4.8, 4.4 and 5.7 for treatments control, enzyme applied to grass forage 24 h before feeding, enzyme applied to grass forage at feeding enzyme applied to barley supplement at feeding, enzyme ruminally infused 2 h after feeding and control [35]. Beauchemin et al. [36] found that Ruminal pH and total VFA concentrations were unaffected by grain source or enzymes treatments, the values were pH 6.03, 6.12, 6.02 and 6.05; VFA, (mM) 103.5, 101.1, 102.5 and 103.0. Ruminal NH_3-N concentration was lower for cows fed the hull-less barley (treated or not treated by enzymes) diets than for cows fed the barley diets. The values were NH_3-N, (mg/L) 124.0, 118.3, 88.1 and 98.4.

The VFA concentration was also numerically higher for cows fed diets containing enzymes than for cows fed the control diet (enzyme vs. control: 130 vs. 122 mM). Proportion of propionate in the total VFA also was numerically higher for cows fed diets containing enzymes than for cows fed the control diet. Consequently, the ratio of acetate to propionate was numerically lower for cows fed diets containing enzymes than for cows fed the control diet. The values of NH_3-N were mg/L 108.3 119.2 105.6 106.9; the pH values were 5.85, 5.88, 5.83 and 5.91 for the control Diet that contained alfalfa hay cubes treated with 1 g of enzyme mixture/kg of hay, a diet that contained alfalfa hay cubes treated with 2 g of enzyme mixture/kg of hay and a diet that contained both alfalfa hay cubes and concentrate treated with 1 g of enzyme mixture/kg of DM [37].

Ruminal fermentation measurements taken pre-feeding showed no differences between enzymatic and non-enzymatic treatment. The enzyme product added to the supplement and the enzyme product added to the premix treatments had differing molar proportions of propiona, but neither treatment differed from the control. The differences in propionate concentration led to differences in the acetate to propionate ratio between these two treatments. The NH_3-N concentration in the rumen at both pre- and post-feeding did not differ between treatments [38].

There were no significant effects on daily mean pH or concentrations of NH_3-N or total VFA although pH was numerically lower and NH_3-N concentration was numerically higher with all the enzyme treatments than with the control. The values were pH 5.88, 5.76, 5.76 and 5.74; NH_3-N, mM 10.1, 11.0, 11.9 and 11.3; Total VFA, mM 129, 129, 129 and 133. For cows fed the control diet; enzyme sprayed on TMR; enzyme sprayed on concentrates and enzyme infused into rumen [28]. ZAD® compound increased rumen pH, rumen TVFA's and NH_3-N concentration for treated animals compare with untreated ones [30].

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The NH_3-N concentration values were high for animals receiving biologically treated sugar beet pulp compared with those fed the control ration. Ruminal TVFA's values showed that treated sugar beet pulp (SBP) was to replace 0% of corn grain included in the concentrate feed mixture and the group that treated SBP was to replace 50% and had higher values of TVFA's at 0, 3 and 6 hours sampling time compared with the group that treated SBP was to replace 75% of corn grain included in the concentrate feed mixture and the group that treated SBP was to replace 100% of corn grain included in the concentrate feed mixture. The pH values for all tested rations are found to be within the normal range [39].

However, Gado et al. [40] showed that NH_3-N concentration reached the maximum after 3 hours of feeding in all treatments. However, NH_3-N concentration were significantly higher in the treated groups than the control group. After 6 h of feeding NH_3-N concentration tended to decrease in all groups. Rice straw treated with fungus maintained the highest value of ruminal total VFA after 3 h of feeding followed by ZAD® treatment, ZAD® with fungus and the lowest values recorded for the control group. The lowest pH values were recorded after 3 h of feeding for the different treatments.

Kholif [32] studied the properties to improve the nutritional value of poor quality roughages (berseem hay, bagasse, wheat straw and rice straw) by using biological treatments such as fungi (*Trichoderma reesei* and *Trichoderma kongei*) and fibrolytic enzymes mixture (1, 2 and 3%). He found that rumen liquor was more acidic when goats were fed on fungal treated silage followed by goats fed on fibrolytic enzyme treated silage compared with the control. Total VFA values were slightly increased with biological treated silage compared with the control. Ruminal total N and true–protein nitrogen were significantly increased with fungal treatment followed by fibrolytic enzyme treatment and then control. Non-protein nitrogen and NH_3-N were not significantly affected by biological treated silage compared with the control. Borhami et al. [41] studded the effect of fungi and ZAD® as biological treatments on the nutritive value of peanut hay. They found that rumen NH_3-N concentration and its rates of production were significantly higher with ZAD® treatments. TVFA were higher with fungi treatments than ZAD® and control treatments.

BEEF AND DAILY GAIN

Bakshi and Langar [42] found that incorporating *Agaricus basporius* in pelleted concentrate mixture either at 20 or 40% (on nitrogen basis) had no significant effect on the daily DMI as g/kg $BW^{0.75}$. Also, in a switchover design study by Ahuja et al. [43], eight crossbreed male sheep of about 15 months old and weighing 50kg were given daily for one month (0.5kg concentrate mixture , 3kg green fodder and 0.5kg spent rice straw (6% CP) after harvesting the mushroom (*Volvariella volvacea*) or 0.5kg untreated wheat straw). Concerning the avarege daily gain (*i.e.*, ADG) and DMI, there were no significant differences between the groups. They concluded that spent rice straw could serve as roughage for sheep. In a 138 day fattening trail, 42 steers (Hungarian pied x Holstein-Friesian) weighing 400kg, were given daily (2kg maize, chopped green maize plant or barely silage *ad libtum* and spent wheat straw after harvesting the mushroom (*Pleurotus ostreatus*) 0, 3, 4.5 or 6 kg in place of hay 3, 2, 1 or 0 kg, respectively. Results obtained showed that final body weight, ADG and DMI of steers were not affected by wheat straw intake [44].

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Deraz [45] found that ADG of ossimi lambs fed biological treated rice straw for 154 days was 106 g/day comparing with those fed control ration (80.06 g/d). The same author reported that feed conversion values were 8.27 kg feed/kg gain for lambs fed biological treated rice straw comparing with 9.88 kg feed/kg gain for the control group. The treated group was more efficient than the control group. On the other hand, Kholif [46] revealed that DMI as g/h/d or g/kg $BW^{0.75}$ was slightly increased for goats receiving rations containing dried banana wastes biologically treated with *Penicillium funiculusms.* However animal weights were not affected by feeding rations containing fungal treated waste.

It was shown in 1960 that feeding a mixture of amylolytic, cellulolytic and proteolytic enzymes (Agrozyme; 1.5, 3 and 6 g/day), as well as a potentproteolytic enzyme (Ficin, Merck and company; 5, 10 and 20 mg/day) did not alter feed conversion or the ADG of fattening lambs fed ground maize or lucerne hay [47]. McAllister et al. [8] have also found that fibrolytic enzymes did not increase DMI or ADG by lambs fed lucerne hay- or barley-based diets. Gado [48] reported that increasing the concentration of cellulase enzyme had a positive reflection on ADG in bagasse treatments in comparison with control treatment. Knowlton et al. [49] found that cows fed diets containing the enzyme formulation gained more weight than those on the control diet. Titi and Lubbadeh [29] reported that there were no significant differences in average birth weights between treated and untreated groups for both awassi lambs and shami kids. The same authors investigated the efficacy of direct feeding a fibrolytic enzyme on lamb digestibility and on the performance of growing milk-type calves. They found that average initial weights were similar for treated and untreated groups in both sexes. Final weights were higher for treated males than controls, while no differences were observed between treated and untreated female calves. Males fed cellulase had higher growth performance than unfed males. Total gain and ADG were higher for the treated males than those of untreated ones. It appeared that fibrolytic enzymes could improve growth performance of dairy male calves under fattening conditions. Zewil [50] found that feeding *ad lib.* biological treated rice straw recorded the highest ADG of 122 g/d while the control ration recorded the lowest value about 65 g/d. Salem et al. [34] found that the ADG and feed efficiency were improved by addition of ZADO®, for sheep and goats fed wheat straw *ad lib.* as a basal diet and commercial concentrate with or without 10g/animal/d of ZADO® and the improvement was more in goats than sheep. Tricarico et al. [33] examined the effects of an *Aspergillus oryzae* extract containing amylase activity on performance and carcass characteristics of finishing beef cattle. They found that α-amylase supplementation increased DMI and ADG. The increases in ADG were related to increased DMI and efficiency of gain during the initial 28-d period but were primarily related to increase DMI as the feeding period progressed. Some studies of the effect of ZADO® or ZAD® are listed in table 2.

IN INDUSTRIAL BY-PRODUCTS

The ZAD® treatment to the experimental by-products decreased the NDF and ADF. It was observed that the amount of degradation in NDF and ADF differed according to type of by-products.

That could be to the geometric structure differences for each fiber content in the tested agriculture by-products. Other reports have also shown increases in DM, particularly fiber,

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digestibility with fibrolytic enzyme addition [12, 52]. Bowman et al. [24], for example, reported a 25% increase in total tract NDF digestibility with a fibrolytic enzyme product, but it appeared that most of the impact was post-ruminal. Cellulases and xylanases usually act synergistically to hydrolyze the forage cell wall [53]. Thus, it is possible that an ideal ratio of endoglucanase and xylanase is needed to enhance the effectiveness of exogenous fibrolytic enzymes [54]. Indeed, exogenous fibrolytic enzymes can access greater surface area compared with cell-bound microbial enzymes. The crystalline regions of cellulose are not easily accessible to endocellulases, whereas the amorphous regions can be attacked by endocellulases and exocellulases [53]. There was an effect of ZAD® on DM digestibility which showed an increased in *in vitro* dry matter digestibility (IVDMD). The highest increase was in bagasse followed by corn stalks and rice straw. The enzyme product evaluated in our study showed higher xynalase than cellulase activity, which could have improved the degradation of alfalfa hay but not of high fiber forages.

Indeed, exogenous fibrolytic enzymes can access greater surface area compared with cell-bound microbial enzymes. The quadratic effects of enzymes on *in vitro* degradation were seen, in general, as the best improvement of *in vitro* digestibility when all tested roughages were treated with 3L of ZAD® for 4 weeks ensiling time.

There was an increase in digestibility coefficient for DM, OM, CP and CF in bagasse, rice straw and corn stalks. Ether extract (*i.e.,* EE) digestibility coefficient was increased only in animals fed treated rice straw. Neutral detergent fiber (*i.e.,* NDF) and ADF digestibility coefficient and total digestible nutrients increased for all three experimental materials.

These improvements on NDF degradation could induce greater DMI [55] by reducing physical rumen fill, increasing the energy density of diets and stimulate microbial N production [56]. A 1% increase in forage NDF *in vitro* degradation has elicited a 0.17kg increase in DMI and a 0.25kg increase in 4% fate corrected milk yield [56]. Thus, the increases in NDF degradation observed in our *in vitro* and *in sacco* assays could potentially increase daily up to 1.0kg of DMI, which might improve the productivity of cattle fed diets contain ZAD® treated agriculture by-products. Digestibility data obtained in the present study are within those obtained in many previous studies [57, 58, 59, 60]. ZADO® activity starts immediately after feeding it to the animals. It works on the microflora directly which reaches its peak after 48h from feeding.

Table 2. Doses and effects of ZADO® or ZAD® on growth performance in ruminants

Enzyme	Doses	Animal	Effects	Ref.
ZADO®	20g/head/day	Goats	Increased ADG by 34%	[20]
ZAD®	3l/ton DM	Goats	Increased ADG by 29%	[48]
ZADO®	40g/head/day	Steers	Increased ADG by16%	[12]
ZADO®	10g/head/day	Sheep	Increased ADG by 41%	[18]
ZAD® +ZADO®	3L of ZAD®/ton DM +5kg ZADO®/ton DM	Sheep	Increased ADG from 130g to 250 g /head/day (sheep fed citrus pulp)	[51]

The main action will be on the rumen kinetics and the improvements on overall performance of the microflora effectiveness on utilizing the feed ingredients that usually reflects on the animal performance of either milk or meat production [14]. It was shown in the

1960 that feeding a mixture of amylolytic, cellulolytic and proteolytic enzymes (Agrozyme; 1.5, 3 and 6 g/day), as well as a potent proteolytic enzyme (Ficin, Merck and Company; 5, 10 and 20 mg/day) did not alter feed conversion or the ADG of fattening lambs fed ground maize or lucerne hay [47].

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IN AGRICULTURAL BY-PRODUCTS

Simple sugars that can be used as fermentable substrates by homolactic bacteria. Therefore fibrolytic enzyme application should make the silage fermentation more homolactic and result in a reduction in proteolysis and DM losses in addition to increasing the digestibility of the forage [61]. There are two primary reasons for adding fiber-digesting enzymes to silage. First these enzymes could partially digest the plant cell walls (cellulose and hemicellulose) yielding soluble sugars which could be fermented by lactic acid bacteria to lower the silage pH.

This would stimulate silage fermentation and improve fermentation quality by increasing the rate and extent of decline in pH, increasing the concentration of lactic acid, improving the lactic acid: acetic acid ratio (which is indicative of greater efficiency of fermentation), and hence reduce DM losses. A faster decline in pH would also limit degradation and deamination of forage proteins and reduce NH_3 production. In addition, partial digestion of the plant cell wall may improve the rate and/or extent of digestibility.

In order to take place the rate of cellulose hydrolysis must coincide with early growth of lactic acid bacteria. For an improvement in digestibility, a change in the association of cell wall components must occur. Jaster and Moore [62] found that haylage pH was 4.4 for the untreated and 4.2 for treated haylage with cellulase and amylase. Kung et al. [63] treated a mixture of barley and hairy vetch with microbial inoculants (1 x 105 CFU/g of wet forage) or cellulase enzyme complex (0.5% of wet weight). They found that microbial inoculant reduced silage pH, acetate and NH_3-N, but lactic acid concentration were increased.

Addition of cellulase enzyme reduced silage pH but did not affect acid or fiber concentration. Eun et al. [31] found that addition of enzyme to untreated rice straw or ammoniated rice straw did not influence total VFA production. Concentration of NH_3-N was higher for untreated rice straw compared to ammoniated rice straw, but it was generally not increased when enzymes were added to untreated rice straw or ammoniated rice straw, except in the case of proteolytic enzyme added to ammoniated rice straw which increased concentration of NH_3-N. Stokes [64] treated second cut crop mixed grass legume with an enzyme mixture containing cellulase, xylanase, cellobiase and glucose oxidase. He found that enzyme treatment reduced silage pH, concentrations of xylose and total sugars and increased titratable acidity and buffering capacity. Furthermore, Fredeen and McQueen [65] reported that the concentration of lactic acid tended to be higher, and the pH tended to be lower, in first cut alfalfa silage treated with enzyme (cellulase, amylase and glucose oxidase). Chen et al. [66] observed that fermented hay crop silage with commercial carbohydrates enzyme (220 ml/ton) significantly reduced silage pH and acetic acid concentration and increased titratable acidity, lactic acid concentration and lactate: acetate ratio. Yang et al. [67] found that Fermenter pH was not affected by the enzyme supplementation regardless of pH level. Total

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VFA concentration was not affected by enzyme supplementation, molar proportion of acetate was higher and that of propionate was lower with enzyme supplementation than with control.

Dean et al. [68] compared the efficacy of four proprietary cellulase, xylanase preparations for improving the digestion and fermentation of five-week growth, Tifton 85 Bermuda grass silage. They found that although all the enzymes had some beneficial effects on the fermentation, one enzyme (Promote, Cargill Corp., St. Louis, MO) proved to be outstanding. This enzyme hydrolyzed the cell walls in the grasses into sugars, which stimulated the growth of homolactic bacteria and resulted in reductions in DM losses, pH, proteolysis and water-soluble carbohydrate utilization, and increased the lactic acid concentration. This enzyme also increased the 6 and 48 h DM digestibility and the 48 h NDF and ADF digestibility of the grass. This study clearly demonstrated that preparations that contain similar enzymes can have different effects on polysaccharide hydrolysis, because of differences in the concentrations and activities of the component enzymes.

Eun et al. [31] found that Total VFA production was higher for ammoniated rice straw compared to untreated rice straw and the addition of enzyme to untreated rice straw or ammoniated rice straw did not influence total VFA production. Molar proportions of acetate decreased when proteases were added to untreated rice straw.

Molar proportions of propionate from untreated rice straw increased by adding all enzyme products, except for cellulases. As a result, adding combination of endoglucanases and xylanases or proteases to untreated rice straw decreased the acetate:propionate ratio.

For ammoniated rice straw, molar portion of acetate decreased with xylanases, xylanases, or proteases, whereas molar portion of propionate increased only when xylanases was added, resulting in a decreased acetate:propionate ratio for xylanases.

Concentration of NH_3-N was higher for untreated rice straw compared to ammoniated rice straw, but it was generally not increased when enzymes were added to untreated rice straw or ammoniated rice straw, except in the case of proteases added to ammoniated rice straw, which increased concentration of NH_3-N. Eun and Beauchemin [54] found that total VFA production from alfalfa hay was increased by feed enzyme products and increased molar proportions of propionate but did not affect the acetate to propionate ratio. Total VFA production from corn silage was not influenced by enzyme treatment.

However, the enzyme treatment generally decreased the molar proportion of acetate and increased the molar proportion of propionate, resulting in decreased acetate to propionate ratio. The molar proportion of butyrate was not affected by enzyme treatments. Giraldo et al. [69] studied the effects of exogenous cellulase supplementation on microbial growth and ruminal fermentation of a high-forage diet in Rusitec fermenters. They found that daily VFA production was increased by 15, 9, and 15% for cellulase from *Trichoderma longibrachiatum*, cellulase from *Aspergillus niger*, and a 1:1 mixture of both cellulases, respectively, with half of the increase being due to production of acetate. All enzyme treatments augmented methane production, but none of them altered the methane: VFA ratio.

BIOLOGICAL TREATMENTS OF FIBROUS FEEDS

The purpose is to allow microbes to degrade cellulose or lignin in residues as a means to improve its nutritive value. Biological methods may include:

4.1. Regular Ensilage

Silage is the material produced by controlled fermentation of crop residues or forages with high moisture content. The purpose is to preserve forages by natural fermentation by achieving anaerobic conditions and discouraging clostridial growth. The ideal characteristics of material for silage preservation are: an adequate level of fermentable substrate (8-10% of DM) in the form of water soluble carbohydrate; a relatively low buffering capacity; and DM content above 200 g/kg.

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The ensiling material should also ideally have, after harvesting and chopping, a physical form that allows easy compaction in the silo. Materials such as maize stover and grass can be ensiled successfully, while crop residues such as rice and wheat straw, with low water soluble carbohydrate content, do not fulfill these requirements, and therefore pre-treatments, such as fine chopping or use of additives, or both, may be necessary.

4.2. Microbial Ensilage

Ensilage of whole fresh maize plants is only practiced for large-scale feedlots and dairy farms. Ensiling dry crop residues after reconstitution of moisture is usually the best way for preserving feeds. Ensiling dry crop residues involves actions such as chopping, reconstitution of moisture, pressing and mixing with certain additives, including micro-organisms such as lactic acid producing bacteria, cellulolytic bacteria, for proper fermentation and nutrient preservation.

A large number of dry crop residues have been successfully ensiled with addition of a microbial. Meng et al. [70] ensiled wheat straw with addition of a specific microbial product containing bacteria that functions as lactic acid and propionic acid producers and cellulose degraders, and fed it to cross-bred steers.

The results showed that microbial ensiling resulted in reduction of NDF, ADF, cellulose and hemicellulose, and an increase in *in situ* DM digestibility. In some feeding studies, it was shown that microbial ensilage of crop residues such as wheat straw, rice straw; maize stover or soybean straw caused increased ADG, DMI and feed conversion, and decreased feed cost per unit gain in growing ruminants.

Several studies [71] also indicated that lactating cows fed diets based on microbial ensiled straw had increased milk and fat-corrected yield, and slightly higher milk fat percentages, compared with diets based on untreated straw.

Another significant effect of microbial ensilage of dry crop residues is probably to hydrate and weaken plant structures so that less energy is expended on rumination. Ensiled crop residues usually have good palatability for ruminants, and thus high intake. In comparison with ammoniated straw, microbial ensiled residues give higher intake, faster rate of passage and therefore better performance. Another advantage of microbial ensilage is its low input cost for acquiring microbial products and accessories, e.g. plastic sheets, and therefore microbial ensilage is considered a better method to enhance the feeding value of dry crop residues.

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4.3. Treatment with White Rot Fungi

Because White Rot fungi can effectively attack lignin and cellulose, their use to treat lignocellulosic material to increase digestibility has been studied quite extensively. Xiao [72] treated wheat straw with strains of *Cyathus stercoreus*, *Bjerkandera adusta*, *Dichomitus squalens*, *Pleurotus* spp. and *Pleurotus ostreatus* for 30 days, and showed that treatment decreased NDF from 71.4 (control) to 67.4, 59.2, 62.7, 65.0 and 67.9%, and ADF from 53.1 to 50.3, 45.1, 46.0, 50.0 and 51.3%, respectively.

After fermentation by the five fungus strains, a considerable loss was found in lignin, from 23 to 44%, and in DM, from 11 to 17%. There was no apparent loss in cellulose and hemicellulose. When wheat straw was incubated *in vitro* with mixed rumen micro-organisms for 24 h, DM digestibility was increased 11 and 8% for the treatment with *Bjerkandera adusta* and *Phleurtus* spp. respectively, compared with the untreated control. Digestibility of straw with the other three strains did not change.

It was also noted that most white rot fungi grew slowly on common crop residues and could not effectively compete against other microbes. Mahrous [73] studied the effect of fungus treatments (*Cheatomium cellublyticum*, *Trichoderma viride* and *Cheatomium cellublyticum* with *Trichoderma viride*) of cotton stalks.

The results were a decrease in dry matter being 91.1, 90.5, 88.1 and 90.0%, respectively. There were substantial increases in CP of the fungal treated cotton stalks against other treatments being 3.7, 10.0, 8.8 and 11.2%, respectively. The ADF content of cotton stalks treated with group *Cheatomium cellublyticum* with *Trichoderma viride* recorded the lower value than those of untreated and treated with group *Cheatomium cellublyticum* or with group *Trichoderma viride*, being 43.1, 45.2, 44.5 and 43.2%, respectively.

These observations suggest that effective breakdown of crop residue cell walls by white rot fungi in practice will require the selection or creation of better strains, and also further refinement of the current treatment techniques.

4.4. Use of Mushroom-Substrate Residues

Crop residues have been used as a substrate to grow mushrooms. The substrate residue after mushroom harvest can be used to feed animals. The most commonly used crop residues are cottonseed hulls, wheat straw, rice straw and maize stover. The residues usually have higher CP and lower CF contents compared with the original substrate.

Research data has also indicated that some species or strains of mushrooms have strong enzymatic activities digesting cellulose and lignin. Regarding the feeding value of this residue, however, more work remains to be done before any overall recommendations can be given.

4.5. Selection of Anaerobic Fungi for Better Fiber Degradation in the Rumen

Filamentous structures of the rumen anaerobic fungi penetrate and substantially weaken the xylem cell walls of forages in the rumen, whereas the bacteria do not adhere to, nor degrade these highly resistant plant tissues to any significant extent. Rumen fungi, but not

bacteria, penetrate the cuticle barrier that protects the leaf's surface. Research indicates that these fungi have unique enzymes, or enzymes with higher specific activities, that give them the ability to weaken and degrade the most limiting structural barriers to degradation. On the basis of these observations it appears that research aimed at promoting the biomass of fungi in the rumen or the establishment of highly active rumen fungi (e.g. by selection) could be a major step forward in increasing the digestibility of poor quality forages by ruminants. The identification of strains of anaerobic fungi that most rapidly degrade the cell walls of forage plants is a necessary prerequisite to the successful inoculation of fungi into the rumen and which will nutritionally benefit the host. Culture, selection of strains and the identification of new species of anaerobic fungi, testing their ability to weaken plant fiber and improving the rate of digestion are worthwhile research areas for development. The research must include developments which allow selected strains to multiply in the rumen and to prevent the competition from wild strains. As rumen fungi are spread by resistant spores [74] which are passed in feces and then, when taken in by the animal in feed, multiply by sporulation in the rumen, the support of specific species to ensure the survival is a research area with a high priority. The selection criteria will include the measurement of the rate of solubilization of fibrous carbohydrates by selected fungi and the weakening of the forage stems following a period of incubation with the forages.

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4.6. Improving the Enzymatic Ability of Rumen Microbes to Degrade Fiber

The concept is simple, if rumen microbes can be developed with greater abilities to degrade fiber, or its components, the microbes will be able to extract a greater amount of the available nutrients from the feed consumed. This often receives a high priority because of the concept that animals fed low quality feeds are primarily energy deficient. In fact, these animals more often are inefficiently using the available nutrients. It must be stressed that the application of balanced nutrition is the first priority and if other disorders of the system are to succeed an animal must have an efficient rumen and also metabolism. However, in an animal fed a roughage diet, with an efficient rumen and balanced for protein, it is possible that for every 5 unit increase in digestibility of the basal roughage resource, there will be 50% increase in ADG on the same basal feed resource [75]. Silva and Ørskov [76] demonstrated that the extent of breakdown of a fibrous feed was dependent on the rumen medium. Untreated ground straw placed in nylon bags, in the rumen of sheep fed ammoniated straw, was fermented to a greater extent than the same untreated ground straw placed in the rumen of sheep fed untreated straw. This difference might be due to a higher concentration of enzymes in the rumen of sheep fed ammoniated compared to untreated straw. The two observations discussed above, when combined, indicate the great potential for improvements in productivity from a successful establishment of a super bug in the rumen, *i.e.*, a micro-organism engineered to produce more, or a greater variety, of enzymes for fiber digestion.

4.7. Enhance the Ability of Rumen Microbes to Degrade Fibrous Feeds

There are potentially three ways to enhance the ability of rumen microbes to degrade fibrous feeds:

1 Selection of microbes, particularly with a high fibrolytic enzyme secretion or that secretes fibrolytic enzymes of high specific activity.
2 Creation of microbes with a greater spectrum of enzyme secretions by recombinant DNA means, e.g. create cellulolytic capacities in xylolytic organisms and vice versa.
3 Create microbes with enhanced fibrolytic activity by recombinant DNA means.

In all cases, the prerequisite will be that the organisms are able to grow in and maintain their space in the rumen. These same prerequisites also apply to the production by genetic engineering techniques of high biological value proteins, other secretions of proteins, amino acids and peptides by rumen organisms.

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4.8. Enzymatic Treatment

All animals use enzymes in the digestion of food, those produced either by the animal itself or by the microbes present in the digestive tract. Therefore, the supplementation of animal feeds with enzymes to increase the efficiency of digestion can be seen as an extension of the animal's own digestive process.

There are four main reasons for using enzymes in animal feed:

1 To break down anti-nutritional factors those are present in many feed ingredients. These substances, many of which are not susceptible to digestion by the animal's endogenous enzymes, can interfere with normal digestion, causing poor performance and digestive upsets.
2 To increase the availability of starches, proteins and minerals that are either enclosed within fiber-rich cell walls and, therefore, not as accessible to the animal's own digestive enzymes, or bound up in a chemical form that the animal is unable to digest (e.g. phosphorus as phytic acid).
3 To break down specific chemical bonds in raw materials that are not usually broken down by the animal's own enzymes, thus releasing more nutrients.
4 To supplement the enzymes produced by young animals where, because of the immaturity of their own digestive system, endogenous enzyme production may be inadequate.

In addition to improving diet utilization, enzyme addition can reduce the variability in nutritive value between feedstuffs, improving the accuracy of feed formulations.

The use of enzymes to attack the lignocellulose structure of crop residues for enhancing their feeding value has been attractive. Crude enzyme products, with cellulolytic and hemicellulolytic capability, are usually added to fibrous feeds in attempts to improve their digestibility. Wang [77] observed that treatment of maize stover with an enzyme product, prepared from *Trichoderma viride*, reduced the contents of some cell wall components and enhanced the ruminal digestibility in sheep. Gado et al. [14] obtained the same results. Commercial cellulase products were also added to diets to increase the supply of readily available carbohydrates. When the enzyme products were included at 0.1-0.2% of the diet of cattle and geese, animal performance was considerably improved. Chen et al. [78] also

reported the use of crude enzyme products prepared from *Trichoderma viride* as feed additives for growing rabbits. In eight growth trials, rabbits fed on a diet with addition of the cellulolytic enzymes gained 17.5-39.3% faster than the control. The difference was consistent and highly significant.

Enzymes exist practically everywhere. They are naturally occurring and are produced by all living organisms and, as nature's catalysts; they speed up the chemical reactions that enable all living things. Without them food could not be digested.

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Enzymes, as with all proteins, are made from chains of amino acids. They speed up or catalyze reactions by binding to their substrate and 'stabilize' the entire reaction process through to product formation, so that far less activation energy is required to move the reaction forwards. As a result, the rate of reaction progression is greatly increased for any given energy status. It is the three-dimensional shape and position of the reactive amino acids within the molecule that confer the catalytic properties of enzymes. Conditions that significantly alter the structure of the active enzyme often result in loss of activity. As a result, enzymes are very sensitive to the environment in which they function and many work best in mild temperatures and mid-range pH.

Henderson and McDonald [79] used two levels of cellulase enzyme to herbage (1 and 4 g/kg fresh grass). They observed that cellulose contents of the silages were significantly reduced after 61 days at the high level of enzyme. In the second experiment, two enzymes produced by *Aspergillus niger* were compared with two enzymes derived from *Trichoderma viride* under the conditions of the experiment. The cellulase enzymes produced from *Trichoderma viride* were more active than those produced by *Aspergillus niger*. Henderson et al. [80] found that treatment with cellulase (4g/kg) resulted in more cellulose being hydrolyzed in the grass silages than in the legume silages. The greatest losses of cellulose in each crop occurred in the minced silages held at 15 and 35^{o}C. Kung et al. [63] treated a mixture of barley and hairy vetch with microbial inoculants (1 x 105 CFU/g of wet forage) or cellulase enzyme complex (0.5% of wet weight). They found that treatments did not affect acid or fiber concentration.

Stokes and Chen [81] reported that an additive enzyme containing, cellulase, xylanase cellobiase and glucose oxidase to whole plant corn reduced NDF and ADF in the first 7 days of fermentation and loss of DM. Silage NDF, ADF, cellulose and hemicellulose were reduced by 11 to 13% and residual water soluble carbohydrate was 20% lower than in control silage. Degradation of structural fiber continued after day 28 of ensilage, but residual water-soluble carbohydrate was not increased. Hoffman et al. [82] observed that enzyme treatment of alfalfa reduced NDF by 3.3% compared with the control silage. Pectic fractions and hemicellulose were reduced by enzyme treatment. Enzyme treated silage contained higher levels of ruminally undegraded NDF and did not alter ruminal DM degradation or cellulose content of alfalfa silage. Sheperd et al. [83] found that addition of the enzyme additive resulted in a linear decrease in ADF, NDF, and hemicellulose content of corn silage but decreased the acid detergent lignin (ADL) content of silage only at the milk and black layer stages of maturity. The enzyme additive had no consistent effect on *in vitro* NDF digestion. Gado [48] studied the effect of supplementing with different concentrations of cellulose enzymes to bagasse silages on their chemical composition. He reported that the effect of the additional different concentration of cellulose enzymes were almost similar on OM, CP, CF and EE. But, it induced a slight decrease for the mentioned components. On the other hand, the degradation various fiber fraction of bagasse increased during the ensiling period with the increasing level

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of cellulose. The NDF, ADF and ADL values of bagasse treated with cellulase at different levels ranged from 70.1 to 71.9%, 61.9 to 62.5% and 11.4 to 11.9% for NDF, ADF and ADL, respectively.

Nadeau et al. [84] treated herbage (orchard grass and alfalfa) with cellulose, combined with inoculant at 2, 10 and 20 ml/kg (at least 2500 IU/ml). Cellulase at 10 ml kg was also applied alone or in combination with pectinase and inoculant or formic acid. The NDF concentration of orchard grass silage decreased with increasing cellulase up to 20ml/kg, at which NDF content was decreased by 30%. The NDF concentration of alfalfa silage decreased with increasing cellulase up to 10ml/kg, at which NDF content was decreased by 13%. Immature plants were more responsive to cellulase treatment than mature plants. Cellulase at 2ml/kg combined with inoculant improved fermentation characteristics of the silages but generally, there was no effect on silage fermentation by higher cellulase applications, resulting in an accumulation of sugar.

Dean et al. [68] studied the effect of fibrolytic enzymes on the fermentation characteristics, aerobic stability, and digestibility of bermuda grass silage; they found that the concentrations of the measured chemical components were similar for all treatments. The DM concentration at harvest was similar to that at which bermuda grass is ensiled. Neither enzyme type nor application rate affected the DM concentration of the silages. The pH of Promote enzyme preparation treated silages was lower than that of all other silages. The loses of DM were lower in the Promote enzyme preparation treated silages than in the other silages. Though there was no effect of increasing pr-application on DM lost, this work demonstrates that Promote enzyme preparation can be used to reduce the losses of DM that usually occur when bermuda grass is conserved as silage. Compared with control silages, NDF concentration was reduced by enzymatic treatments.

Gado et al. [40] found that the rice straw treated with ZAD®, rice straw treated with fungus and rice straw treated with ZAD® and fungus decreases DM. A substantial increase in CP of the fungal treated rice straw against other treatments was found being 9.79, 6.08, 8.07 and 3.41% for ZAD®, fungus, ZAD® treated with fungus and control, respectively. Kholif [32] studied the properties to improve the nutritional value of poor quality roughages (berseem hay, bagasse, wheat straw and rice straw) by using biological treatments such as fungi (*Trichoderma reesei* and *Trichoderma kongei*) and fibrolytic enzymes mixture (1, 2 and 3%). He found that fungal and enzyme treatments increased CP and decreased CF contents of silages. *Trichoderma reesei* recorded the highest value of CP content compared with treatments and the lowest value of CF content of fungal treatments. While, the enzyme mixture (2%) recorded the highest value of CP content for enzyme levels treatments and recorded the lowest value of CF content OM, EE, and NFE were slightly decreased with treatments compared with control.

Eun and Beauchemin [54] assessed the effects of 4 feed enzyme products that varied in enzymatic activities on the degradation of alfalfa hay and corn silage. The feed enzyme contained a range of endoglucanase, exoglucanase, xylanase, and protease activities, and a range of dose rates was used. They found that quadratic increases in gas production and degradation of DM and fiber were observed for all feed enzymes, with maximum responses at low to medium dose rates. For corn silage, none of the feed enzymes increased gas production or DM degradation, but all feed enzymes increased NDF degradation, with the optimum dose. Eun et al. [85, 86] studded the use of exogenous fibrolytic enzymes to enhance *in vitro* fermentation of alfalfa hay and corn silage. They found that exogenous fibrolytic enzymes

substantially improved gas production and degradation of alfalfa hay and corn silage fiber. The optimum dose rates of these exogenous fibrolytic enzymes was 1.4 mg/g of DM for both forages with improvements in NDF degradability up to 20.6% for alfalfa hay and up to 60.3% for corn silage. Whereas added activities of endoglucanase and exoglucanase were positively correlated with improvement in NDF degradability for alfalfa hay and corn silage, there was no relationship between added xylanase activity and NDF degradability.

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CONCLUSION

1- The potential of enzymatic action on agriculture by-products is valid.
2- The animal response in dairy or beef production is very clear and strong.
3- The improvement in animal production is about to enter a new dynasty and research in this area will benefit of all human kind.

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Chapter 2

ANTIBIOTICS IN ANIMAL NUTRITION

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ABSTRACT

Antibiotics are natural or synthetic chemical substances that block the growth of cells. Antibiotics, specifically antimicrobial drugs, are commonly used in food-producing animals to treat, prevent and control disease, as well as to improve growth and feed efficiency. The exact mechanisms by which antibiotics improve these parameters are not well understood, but may include suppression of subclinical infections, changes in the abundance and/or composition of the intestinal microbiota, reduction of growth-depressing microbial metabolites in the intestinal tract, reduction of nutrient utilization by intestinal microorganisms, as well as increased nutrient uptake through a thinner intestinal wall.

The overuse of antibiotics in animal farming is thought to have led to an increased prevalence of antibiotic resistance in several pathogens, some of which can represent a threat to human welfare. The potential association between antibiotic use in animal farming and a spread of pathogens with antibiotic resistance to human populations has prompted the use of other types of feed additives, such as probiotics, prebiotics or a combination of both. The economic impact of banning subtherapeutic use of antibiotics is huge, an estimate in 2001 calculated $242.5 million in swine production in the United States, with consumers sharing about 75% of these costs in the long run. More efficient strategies are needed to meet the nutritional needs of an always growing human population while at the same time avoiding and/or preventing the spread of multiple drug resistance in pathogenic organisms.

The study of metabolic pathways for antibiotics in the host and the resulting consequences for human populations can help design better strategies to be used in farm animals.

ANTIBIOTICS IN NONRUMINANTS

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Introduction

Antibiotics are substances that block the growth of cells. Many antibiotics are chemical compounds naturally synthesized by soil microorganisms, such as *Streptomyces* spp., which help them compete and survive against other naturally occurring soil microorganisms. Many antibiotics stop the growth of bacteria by specifically blocking DNA replication. Because several components of the replication machinery have remained relatively unchanged throughout evolution, many of these antibiotics work against essentially all types of bacterial organisms. In addition, some are effective against eukaryotic cells and therefore are used as antifungal agents and in tumor chemotherapy. Some examples of antibiotics that block DNA replication include trimethoprim and nalidix acid, which are chemically synthesized, and novobiocin and mitomycin C, which are naturally synthesized by *Streptomyces* spp. Other antibiotics affect DNA structure, such as the acridine dyes proflavine, ethidium, and chloroquine, which insert themselves between the bases of DNA and thereby cause frame shift mutations, thus inhibiting the synthesis of DNA [1].

The discovery of the growth-enhancing properties of antibiotics can be considered one of the most important discoveries of the past century. This effect was reported for the first time by Moore *et al.* [2], since when it has been extensively applied in the US and abroad, bringing a considerable increase in livestock production. The original work by Moore was confirmed when Stokstad and Jukes showed in 1950 [3] that pure aureomycin hydrochloride would increase the rate of growth of the young turkey. Similar results were reported in pigs and confirmed by many other researchers [4]. Shortly afterwards, numerous articles were published and several animal farming industries adopted the use of antibiotics in feeds as a very efficient way for increasing their economic return [5].

In pig production, the supplementation of antibiotics in the food is widely practiced around weaning, a period in which the piglets are removed from the mothers. Weaning is one of the most critical periods for piglets due in part to its immature gastrointestinal tract. In part due to a growing public concern about potential transference of antibiotic resistance from animal products to humans, an increasing number of antimicrobial growth promoters have been banned in developed countries. As a result of this ban, a growing number of reports suggest higher incidences of subclinical diseases and a decrease in animal performance.

Mechanism of Action

Published research data have shown that the use of antibiotics in animal farming can improve the rate and efficiency of body weight gain, reduce mortality and morbidity, reduce subclinical disease, and maintain an optimum health status in pigs. Also, the use of antibiotics at breeding and during lactation can benefit reproductive and lactating performance in sows.

The economic benefits are several-fold greater than the cost of the antibiotic when a cost-effective antibiotic is used for this purpose [6].Although not very well understood, several mechanisms by which antibiotics lead to an improvement in growth performance of animals have been proposed [7]:

- Suppression of subclinical infections.
- Changes in the abundance and/or membership composition of the intestinal microbiota.
- Reduction of growth-depressing microbial metabolites (e.g. toxic amines).
- Reduced utilization of nutrients by microorganisms.
- Increased nutrient uptake through a thinner intestinal wall.

Also, a low, continuous dose of antibiotic may:

- Eliminate bacteria that steal essential nutrients required by the animal for growth.
- Reduce competition allowing beneficial bacteria that produce essential nutrients required by the animal for growth to multiply.
- Control growth of bacteria that cause low grade infections or produce toxins, both of which result in thick intestine that do not absorb nutrients well.

Some antibiotics may also enhance feed consumption and growth by stimulating metabolic processes within the animal. Also, antibiotics may elicit part of their animal growth-promoting effect by decreasing the stimulation of the immune system, allowing more nutrients to be partitioned away from an immune response and toward lean tissue accretion[8].

The effect of antimicrobial drugs in growth performance of food-producing animals has been demonstrated; however, contradictory results are common in the literature. For example, Maxwell *et al*. [9] compared the effect of three different antibiotics on weight gain, feed efficiency and daily feed intake in pigs, and showed that all these parameters were essentially the same across all treatment groups when compared to an untreated control group (Trial 1, n=80 pigs). In a second trial (n=498), the same researchers showed that pigs fed virginiamycin during the growing period (18 to 53 kg) grew faster and were more efficient than those receiving chlortetracycline ($P<0.05$). However, pigs fed virginiamycin grew faster ($P<0.05$) but only tended ($P<0.1$) to be more efficient than pigs fed the non-medicated control diet, thus questioning the effect of the addition of the antibiotic in the diet when compared to a regular diet without the antibiotic.

More recently, Weber *et al.* [8] studied the effect of antibiotics on daily gain weight using weanling pigs (initial body weight 7.6 kg and 29 days of age). The pigs were fed experimental diets, either medicated or no medicated, for 9 weeks. Medicated diets fed during weeks 1 through 3 contained 55 mg carbadox/kg; during weeks 4 through 6 contained 299 mg tilmicosin/kg; and during weeks 7 through 9 contained 110 mg tylosin and 110 mg sulfamethazine/kg. Thereafter, all pigs were fed identical medicated diets for the duration of the finishing phase.Pigs fed medicated diets had higher overall average daily gain than pigs fed unmedicated diets for weeks 0 through 9. Feed efficiency was greater for pigs fed medicated diets than for pigs fed unmedicated diets for the duration of the nursery phase

(weeks 0 through 4 of the experiment). However, there were no significant effects of antibiotics on average daily feed intake, or adjusted days to 114 kg of body weight. Average daily gain for weeks 9 to 17 in the finishing phase was not affected by previous antibiotic treatments [8].

Walsh *et al*. [10] reported that 19-days old weanling pigs fed during 34 days diets added with 55 ppm of carbadox (CB) had 11% greater average daily gain (389 vs. 348 g/day), 8% higher average daily feed intake ($P < 0.007$, 608 vs. 559 g/day), and were 8% heavier on day 34 of the experiment ($P < 0.001$, 18.8 vs. 17.3 kg) than pigs fed either diets without antibiotics or acidified diets (basal diet + 0.4% organic acid-based blend (fumaric, lactate, citric, propionic, and benzoic acids; Kemin Americas)) on days 0 to 7; followed by 0.2% inorganic acid-based blend (phosphoric, fumaric, lactic, and citric acids; Kemin Americas) on days 7 to 34. In a second experiment, 18-days old weanling pigs fed diets added either with 55 ppm carbadox or with 38.6 ppm of tiamulin + 441 ppm of chlortetracycline on days 0 to 7, followed by 110 ppm of chlortetracycline on days 7 to 35, had greater average daily gain ($P < 0.004$; 315 and 303 vs. 270 g/d, respectively), average daily feed intake ($P < 0.01$), and body weight at day 35 of the experiment ($P < 0.002$; 16.7 and 16.2 vs. 15.1 kg, respectively) than pigs fed diets without antibiotics [10].

Another study showed that the antibiotic virginiamycin (VIR) added at a level of 11 mg of VIR/kg of diet, improved both calcium (54.99 to 58.30%, $P = 0.002$) and phosphorus digestibility (32.71 to 37.72%, $P < 0.001$) in growing pigs. The increase in digestibility was not as great as that provided by phytase enzyme, with its direct effect on feed. However, two additional antibiotics, (bacitracin methylene disalicylate and tylosin) did not result in an improvement in phosphorus, calcium, and dry matter digestibility by growing pigs. Thus, improvements in phosphorus digestibility seem to be an antibiotic-specific response rather than a generalized antibiotic response [11].

Changes in the Microbiota

One suggested mechanism by which antibiotics can enhance growth in animals is by changing the abundance, metabolism and/or membership composition of the intestinal microbiota. Indeed, oral administration of antibiotics is likely to change the composition of the gut microbiota, which in turn may favor growth in the host. Nonetheless, debate is ongoing as to what represents a "normal" microbiota in clinically healthy, untreated individuals, as well as to how that normal microbiota is changed. Also, a change in the microbiota may simply be a reduction in numbers, a change in species composition or a combination of the two. Because of methodology barriers, this phenomenon was not investigated in depth until recently. Rettedal *et al.* [12] studied the effect of chlortetracycline on the ileal microbiota of piglets using a cultivation-independent approach based on DNA extraction, PCR amplification, cloning, and sequencing of the 16S rRNA gene. In this study, chlortetracycline treatment resulted in three major shifts: a decrease in *Lactobacillus johnsonii*, an increase in *L. amylovorus*, and a decrease in *Turicibacter* phylotypes. More recently, Looft*et al.* [13] used phylogenetic, metagenomic, and quantitative PCR-based approaches to compare the effect of a combination of three different antibiotics on the fecal microbiota of piglets, and showed that medicated pigs (n=3) had an increase in members of the phylum Proteobacteria (specifically *E. coli* organisms) compared with nonmedicated pigs

(n=3) at the same time point. Interestingly, the authors also analyzed the fecal microbial metagenome (i.e., all the microbial genetic material) and showed that microbial functional genes relating to energy production and conversion were increased in the antibiotic-fed pigs. The results also indicated that antibiotic resistance genes increased in abundance and diversity in the medicated swine microbiome, despite a high background of resistance genes in nonmedicated swine [13].

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Health Concerns

In industrialized countries, epidemiological data has suggested that a significant source of antibiotic resistant food borne infections in humans may be the acquisition of resistant microorganisms from animals via food. However, the magnitude of the public health risk associated with antimicrobial use in animals has been hotly debated for more than 30 years. A number of pathogenic bacteria and other microorganisms are remarkably resilient and can develop ways to survive after exposure to drugs meant to kill them. This antibiotic resistance, also known as antimicrobial resistance or drug resistance, is thought to be due to the excessive use of antibiotics. Since the middle 90's, the U.S. Food and Drug Administration (FDA) have been involved in several initiatives to diminish the unnecessary use of antibiotics in animal farming. For example, recently in April 5, 2012, the FDA issued an order that prohibits certain uses (e.g. for disease prevention) of the cephalosporin class of antimicrobial drugs in cattle, swine, chicken, and turkeys. Since the last twenty years of the 20^{th} century and up to now, there has been increasing pressure by some civil groups to eliminate the use of subtherapeutic antibiotics in animal feeds mainly because of perceived risks posed to human health by animal agriculture via the emergence of antibiotic-resistant bacteria [5, 14].

The use of fluoroquinolones and the development of resistant *Campylobacter* spp. in chicken are of special concern, since *Campylobacter* has been reported to develop resistance when fluoroquinolones are used and epidemiological research on sporadic infections indicates that chicken is the most common source of human infection.

Although the incidence of antibiotic resistance in the human population remains high, there is no clear evidence whether or not antibiotic usage in swine, poultry, and other food-producing animals contributes to antibiotic resistance in the human population. One rationale in favor of this is that even though antibiotics have been fed for nearly 50 years to literally trillions of animals, there is still no convincing evidence of unfavorable health effects in humans that can be directly linked to the feeding of subtherapeutic levels of antibiotics to swine or other animals [6].

Repeated reports from recent years, indicating new findings of resistance by *Enterococcus*, *Salmonella*, *Campylobacter* spp. and *Yersinia enterocolitica* against the antibiotics Monensin-Na, Salinomycin-Na, Avilamycin and Flavophospholipol led to the European Union to a general ban of all the antibiotics used as growth promoting agents [15]. Bhandari *et al*., [16] stated that, enterotoxigenic *Escherichia coli* is a major problem in the swine industry and results in scouring, increased mortality, and poor performance in the period immediately postweaning. Although the traditional way to control this problem is to include subtherapeutic antibiotics in the feed (for example 110 mg/kg of chlortetracycline, 110 mg/kg of sulfamethazine, and 55 mg/kg of penicillin), this is no longer

acceptable to many consumers; thus, alternatives to antibiotics are being searched and some examples are animal blood products produced from the rendering process like spray-dried porcine plasma (SDPP), *Bacillus subtilis* direct-fed microbial (DFM), blends of organic acids, and sweeteners.

Table 1. Some antibiotics commonly used in the United States

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Active ingredient	NADA/ANADA	Label type	Indications
Chlortetracycline.	N046-699	Type C	Breeding swine: For control of leptospirosis cause by *Leptospira pomona* susceptible to chlortetracycline.
Neomycin sulfate and oxytetracycline.	N138-939	Type C	Breeding swine: For control of leptospirosis cause by *Leptospira pomona* susceptible to oxytetracycline.
Ractopamine and tylosin.	N141-172	Type B	Finishing swine: For increased rate of weight gain, improved feed efficiency and increased carcass leanness
Chlortetracycline.	N046-699	Type C	Growing swine: For an increased rate of weight gain and improved feed efficiency
Virginiamycin.	N091-467	Type C	Swine: For an increased rate of weight gain and improved feed efficiency; as an aid in the control of swine dysentery.
Diclazuril and Roxarsone.	N141-223	Type C	Broiler chickens: For increased rate of weight gain, improved feed efficiency and improved pigmentation.
Lasalocid and bambermycins.	N141-129	Type C	Broiler chickens: For increased rate of weight gain and improved feed efficiency.
Oxytetracycline.	N138-938	Type C	Broiler chicken: For increased rate of weight gain and improved feed efficiency; for reduction of mortality due to air sacculitis caused by *Escherichi coli* susceptible to oxytetracycline.

Source: [18].

Currently Used Antibiotics

The number, types and amounts of antibiotics that are included as food additives is somewhat regulated by federal governments. In 2005, only few antibiotics were approved by Health Canada for growth improvement in livestock: chlortetracycline, virginiamycin, bacitracin, bambermycins, lincomycin, salinomycin, penicillin, monensin, tylosin, and lasalocid.

According to the most recent report of the USDA Animal and Plant Health Inspection Service [17], the three antibiotics most commonly used in diets for grow-finish pigs in the US are chlortetracycline, tylosin, and bacitracin [11]. However, in the US and other countries other antibiotics are also commonly used (Table 1) depending on the animal species and the stage of production.

Other Animal Species

Another animal species where antibiotics have been used extensively is the chicken. Many antibiotics are used in the chicken to increase rates of weight gained, prevent coccidiosis, improve feed efficiency, as well as to improve pigmentation, including salinomycin sodium, chlortetracycline, diclazuril, roxarsone, lasalocid, bambermycins, narasin, bacitracin zinc, nicarbazin and bacitracin methylene disalicylate, oxytetracycline, virginiamycin.

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Some of these compounds are also used in turkeys for the same purposes.

The use of antibiotics to promote growth has been mainly done in food-producing animals, such as pigs and chicken.

In other animal species, the use of antibiotics aims to cure disease. Little information is available in the FDA website for aquatic species such as catfish, lobster, and salmonids. Interestingly, a growing number of investigations are shedding light into the effect of antibiotics on the intestinal microbiota of common pets, such as the dog and the cat [19]. Recent studies suggest that the consumption of chlortetracycline may lead to changes in the microbial metabolism of the rabbit cecum.

Use of Antibiotics in Cattle

The raising of cattle for meat and milk has become an integral component of the food production systems due to the vast increase in population in the world. Antimicrobial compounds are a critical component of food-animal production. They provide many benefits related to animal health, animal welfare, and economic return for the industry. Since the discovery of the benefits of the utilization of antibiotics which mainly promoted animal growth and feed efficiency, the number and use of these products has increased [20].

It is certainly recognized that the inclusion of antimicrobials in the diet of livestock improves growth rates. Since the mid forties, when it was acknowledged that antibiotics improved animal growth responses, a substantial amount of studies have been conducted in all species to discern its mode of action. The beneficial effect of antibiotic growth promoters is based on their capability to manipulate the microbial flora of the intestinal tract of most species as well as the rumen microbiota. This compound-microbe interaction results in improved digestion, metabolism and absorption of carbohydrates, proteins, minerals and vitamins.

Moreover, as a result of an enhanced nutrient utilization, reduced intake and thus diminished waste is promoted in supplemented animals [21]. The incorporation of antibiotic growth promoters to current nutritional practices offers significant advantages (Table 2).

In cattle, while increasing the efficiency of nutrient utilization, the demands of feed intake are diminished, moreover, improved efficiency also results in reduction of wastes and diminished adverse environmental impact.

Antibiotic utilization also promotes a more stable rumen fermentation preventing metabolic disorders. Finally, animal health and welfare is supported due to an improved immune status and precluded enteric diseases.

Table 2. Effects of antibiotic growth promoters in animals

Effect	change	Effect	Change
Feed intake.	↕	Plasma nutrients.	↑
Ammonia production.	↓	Beneficial bacteria.	↑
Gut wall diameter.	↓	Adverse bacteria.	↓
Gut energy loss.	↓	Transferable resistance.	↕
Toxic amine production.	↓	Competition for nutrients by gut flora.	↓
Gut wall length.	↓	Nutrient synthesis by gut flora.	↑
Alpha-toxin production.	↓	*Clostridium perfringens.*	↓
Gut wall weight.	↓	Pathogenic *E. coli.*	↓
Fatty acid oxidation.	↓	Pathogenic streptococci.	↓
Faedcal fat excretion.	↓	Beneficial lactobacilli.	↑
Faecal moisture.	↓	Benefical E. coli.	↑
Mucosal cell turnover.	↓	Debilitation of pathogens.	↑
Stress.	↓	Nitrogen excretion.	↓
Gut urease.	↑	Phosphorus excretion.	↓
Gut food transit time.	↑	Methane emission.	↓
Energy retention.	↑	Liver protein synthesis.	↑
Nitrogen retention.	↑	Trace element absorption.	↑
Limiting amino acid supply.	↑	Gut alkaline phosphatase.	↑
Gut absorptive capacity.	↑	Fatty acid absorption.	↑
Vitamin absorption.	↑	Glucose absorption.	↑
Vitamin synthesis.	↑	Calcium absorption.	↑

Adapated from: [22].

Mode of Action of Antibiotics that PromoteGrowth

The precise mechanism of action of antibiotics in improving growth and enhancing feed efficiency in ruminants is rather complicated and is the result of their intrinsic antimicrobial activity and other actions. Nonetheless, it has been accepted that the growth response is due primarily to actions on the rumen microbiota to improve fermentation efficiency [21].

The mechanism of action of the main antibiotics used in cattle are included in the following three categories:

Table 3. Major antimicrobials used in cattle

Generic name	Class	Mode of action
Bambermycin	Polypeptide	Cell wall inhibitor
Lasalocid	Ionophore	Ion exchange promoter
Monensin	Ionophore	Ion exchange promoter
Narasin	Ionophore	Ion exchange promoter
Salinomycin	Ionophore	Ion exchange promoter
Oleandomycin	Macrolide	Ribose function inhibitor
Tylosin	Macrolide	Ribose function inhibitor
Virginiamycin	Macrolide	Ribose function inhibitor

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1. Ribosome function inhibitors ,
2. Cell wall inhibitors, and
3. Ionophores (Table 3).

RIBOSOME FUNCTION INHIBITORS

The macrolide class of antibiotics consists of natural products isolated from fungi and their semi-synthetic derivatives. The macrolide structure is characterized by a 12-16 atom lactone ring; however, none of the 12-member ring macrolides are used clinically. The antimicrobial activity of the macrolides is attributed to the inhibition of protein synthesis. Macrolides bind to the 50S subunit of the ribosome, resulting in blockage of the transpeptidation or translocation reactions, inhibition of protein synthesis, and thus the inhibition of cell growth [23]. These compounds are active against most aerobic and anaerobic Gram-positive bacteria [24].

Tylosin is a widely used antimicrobial in a variety of livestock production systems. It is a macrolide which is active against some Gram-negative bacteria (including *Campylobacter* and *Pasteurella* species) and has a useful activity against certain mycoplasmas.

In addition, tylosin is specifically indicated in cattle for prophylaxis against hepatic abscesses caused by *Fusobacterium necrophorum* and *Actinomyces pyogenes*. Hepatic abscesses in feedlot cattle are a serious economic problem due to the fact that carcass yields, fat thickness and USDA yield grade are diminished as a result of liver abscessation.

Nagaraja *et al*. [25] indicated daily gain and feed efficiency reductions as much as 11 and 29.5%, respectively, as a result of hepatic abcesses in cattle. Tylosin has been widely used in conjunction with other antibiotics in beef cattle studies [26, 27, 28]. Some other antimicrobials also used to counteract hepatic abscesses are bacitracin and virginiamycin.

Viginiamycin is produced by a mutant strain of *Streptomyces virginiae*. It is composed of two major factors, M and S that function synergistically, where M blocks the elongation of polypeptide chains by binding poptidyl transferase, and S, which has a similar action, enhances and fixes the bound components.

Virginiamycin is an important tool in preventing a range of diseases in animals, it is also used as a feed additive to improve daily live weight gain and feed conversion efficiency in feedlot and grazing cattle [29].

Virginiamycin acts by altering ruminal microbial populations promoting changes in fermentation products, namely a reduction in ammonia and amine production as well as an increase in propionate at the expense of acetate and methane As the metabolites represent potential losses, a reduction in their production results in increased availability of protein and energy for use by the host [30].

Oleandomycin is a macrolide antibiotic produced by *Streptomyces antibioticus*. It is widely used to promote growth in food producing animals [31, 32].

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CELL WALL INHIBITORS

Bambermycin is a glycolipid antibiotic produced by *Streptomyces* species including *S. bambergiensis*, *S. ghanaensis*, *S. geysirensis*, and *S. ederensis*. The main component of bambermycin is a phosphorus-containing glycolipid known as moenomycin A. Its mode of action is based on the inhibition of peptidoglycan synthesis, specifically by blocking the formation of the murein polysaccharide strands. Bambermycin is used only as a growth-promoting antibacterial in animal feeds. Gram-positive microorganisms are sensitive to bambermycin, although it also exhibits little activity against certain gram-negative bacteria, such as *Pasteurella* and *Brucella* [33].

IONOPHORES

The Rumen Environment

The rumen-reticulum fermentation vat constitutes an extremely complex microbial ecosystem, which involves the interaction of billions of microorganisms per ml of rumen fluid consisting of anaerobic bacteria, ciliated protozoa and fungi. Nonetheless, through a number of different physiological mechanisms, the host animal maintains a rather constant environment within the rumen. The steady supply of food and continuous removal of microbial fermentation products (VFA, CO_2 and CH_4) and food residues, provides ideal conditions for the development and growth of the rumen microbiota.

Animal performance is dependent on the balance of these interactions. The VFA are used by the animal as an energy source, while the microbes serve as an important source of amino acids for protein synthesis. Ammonia, methane and heat, by contrast, represent losses of either nitrogen or energy to de the animal [34].

Microbial energy transformations affect all aspects of microbial ecology, and hence ruminant nutrition. They determine not only the quantity and composition of the fermentation acids, but the amount of biomass present. Since the rates of feedstuff fermentation and degradation depend on the cell density, and since the animal is dependent on microbial protein as an amino acid source, the energy metabolism of the rumen microorganisms affect both the energy and nitrogen status of the animal.

Not surprisingly, the complex rumen ecosystem is challenged by exposure not only to different diets but also to antibiotics. Significant changes in the relative abundance of the resident microbiota lead to changes in net fermentative function. Depending on the effect of

this change on energy transformation, the outcome may be improved in the retention of energy, a commonly encountered occurrence with the use of antibiotic growth promoting antibiotics [22].

While rumen fermentation efficiency depends on the nature of the diet, it is critically determined by the composition (qualitative and quantitative) of its microbial population. By manipulating the composition of this complex ecosystem, feed additive as polyether ionophore antibiotics can significantly modify energy and protein digestion [35].

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MODE OF ACTION OF IONOPHORES

The development of a range of methods of enhancing growth in farm animals has resulted as an effort to improve productivity in livestock. Normally occurring metabolites of certain molds and bacteria with antibiotic action have been used to promote growth in livestock production systems [36]. Polyether antibiotics belonging to the valinomycin class of ionophore antibiotics (e.g. lasalocid and monensin, Figure 1, monensin), were initially developed as coccidiostats for poultry; however, later research demonstrated their effectiveness on ruminal fermentation which supported their utilization as growth promoters in cattle.

Ionophores are molecules with structural backbones that contain strategically spaced oxygen atoms. The backbone is capable of assuming critical conformations that focus these oxygens about a ring into which a complexiblecation may fit more or less snugly [37].

The ionophores interfere with the natural ion transport systems of both prokaryotic and eukaryotic cells. Ionophores lower the energy barrier necessary for the trans membrane transport of ions and catalyze an electroneutral cation-proton exchange across the barrier. Consequently, they abolish the gradients of Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, causing cell death [23].

The carboxylic ionophores form cationic complexes only in their protonated anionic form. Ideally, they carry anions as electrically neutral zwitterions; however, they do have minor modes of transporting current as multimoiety complexes. They are capable of carrying protons on their protonated carboxyl form.

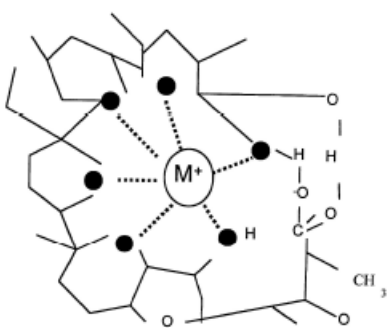


Figure 1. Molecule of the ionophore monensin [37].

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The cycle of a carboxylic ionophore –catalyzed cation-for-proton-exchange is illustrated in Figure 2, where:

1. Protonated ionophore within a membrane diffuses to one interface.
2. Release of proton; the ionophore is trapped at the polar interface due to increased polarity of charged anionic form.
3. Ionophore anion encounters a complexible M+, which is engulfed and its water of salvation displaced.
4. Zwitterionic complex break away from the interface and difusse to the opposite interface.
5. Release of cation coincident with resolvation
6. Ionic ionophore combines with a proton: it lowers its polarity and permits the ionophore to leave the interface and return to the membrane interior to start another cycle.

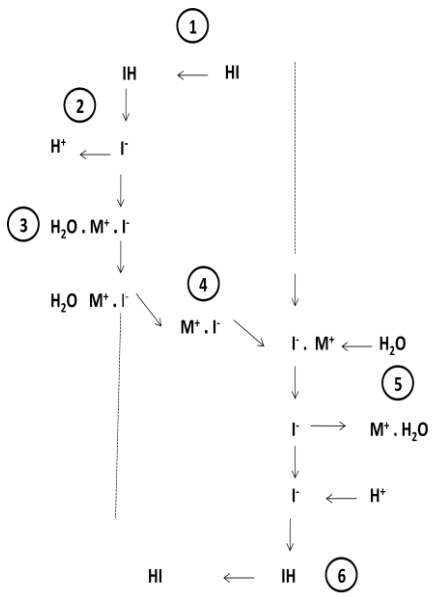


Figure 2. Cycle of a carbocylic ionophore indicating the proton exchange [Adapted from 37].

Monensin is the polyether ionophore most extensively used, although some others; e.g. lasalocid, maduramicin, monensin, narasin, salinomycin, and semduramicin (Table 4), are also used in veterinary medicine for their antibacterial and anticoccidial activities [24, 33].

Table 4. Polyether ionophores antibiotics used in animals

Ionophore	Fermentation product of	Year of discovery	Empiricalformula
Lasalocid	*Streptomyces lasaliensis*	1951	$C_{34}H_{54}O_8$
Monensin	*S. cinnamonensis*	1967	$C_{36}H_{62}O_{11}$
Salinomycin	*S. albus*	1972	$C_{42}H_{70}O_{11}$
Narasin	*S. aureofaciens*	1975	$C_{43}H_{72}O_{11}$
Maduracin	*Actinomadura yumaense*	1983	$C_{47}H_{80}O_{17}$
Semduramicin	*A. roseofera*	1988	$C_{45}H_{76}O_{16}$

[Adapted from 22, 24].

EFFECT OF IONOPHORE ANTIBIOTICS ON PRODUCTION

Ionophores are commonly used for improving production efficiency by altering the gastrointestinal microflora of animals [21]. Ruminal fermentation is inherently inefficient, with the conversion up to ≤ 12% of dietary carbon and energy to methane and heat that are unusable for the animal and maximum ≤ 50% of dietary protein is degraded to ammonia and lost in the urine.

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Ionophore induced improvements in productivity resulting from changes in the population of VFA produced during ruminal digestion, supporting the fact that microbial energy transformations affect all aspects of microbial ecology, and hence ruminant nutrition. Monensin addition to diets results in improvements in live weight gain up to ≤ 10%, increases in feed conversion of around ≤ 7% and decreases in intake of proximately ≤ 6% [22, 24].

IONOPHORES AND ITS USE IN PREVENTING METABOLIC DISORDERS

The benefits of the antimicrobials now extend well beyond a direct improvement in daily gain and feed efficiency. Nowadays, there is a concise evidence that ionophore promote optimal and stable fermentation patterns that can avert the adverse consequences of several metabolic diseases in ruminants. Altered ruminal fermentation associated with ionophore feeding has been shown to be preventive against certain digestive disorders in ruminants such as bloat and acidosis [21, 22].

BLOAT

The rumen of mature cattle can produce 1.2 – 2.0 liters of gas per minute [38]. The gas is the product of rumen fermentation and is composed primarily of carbon dioxide and methane. Any factor that interferes with eructation can produce bloat, which is a serious, frequently lethal condition of cattle consuming large quantities of legumes or in feedlot fed high concentrate diets.

The primary factor in this common form of bloat is a change in the ruminal contents to a foamy or frothy character because of altered surface tension. The chemical changes that cause foam to form within the rumen are not fully understood, nonetheless, some researchers have indicated that foam producing agents are of microbial origin, while others indicated that some plant factors involved in bloat such as plant pectin and pectin methyl esterase as well as a soluble legume protein fraction with ribulose diphosphate carboxylase [39]. Although management practices must be implemented to reduce impact of nutrition in ruminal bloat, additional measures are needed to achieve an acceptable balance of health and growth rate. Ionophore antibiotics generally do not eliminate the bloat but cause a relevant diminish in the incidence [40, 41]. The ionophore monensin has been used in controlled-release capsule which is introduced into the rumen of cattle grazing legume pastures to control frothy bloat [42, 43].

LACTIC ACIDOSIS

Lactic acidosis occurs in cattle when they are allowed access to large amounts of readily fermentable carbohydrates. *Streptococcus bovis* and *Lactobacillus spp* which are Gram-positive bacteria, are considered to be chiefly responsible for rapid fermentation and production of large quantities of lactic acid. When lactic acid accumulates more rapidly than it is absorbed, rumen pH falls and rumen atony develops resulting in some cases lethal in less than 24 hours. Management practices have to be implemented to ensure that intake carbohydrate intake is well controlled [44].

The ionophore antibiotics possess ideal characteristics for preventing lactic acid build-up in the rumen. Because of their selectivity toward Gram-positive bacteria, major lactic acid-producing ruminal bacteria (*S. bovis* and *Lactobacillus* spp.) are inhibited, but ruminal lactic acid-fermenting bacteria, being Gram-negative, are unaffected [45]. Cattle subjected to experimental acidosis and treated with ionophore antibiotics have maintained higher ruminal pH and lower lactate concentration than controls [46].

ENVIRONMENTAL BENEFITS OF THE USE OF ANTIMICROBIALS IN CATTLE

Methane

There is considerable concern about the impact of agriculture – and meat production in particular – on land use, energy, and greenhouse gas emissions. The greenhouse gases of greatest interest in livestock are methane, carbon dioxide and nitrous oxide which are environmental hazards. Methane emitted from the livestock sector contributes significantly to greenhouse gas [47].

Methane released to the atmosphere by livestock represents 40% of the emissions produced by human related activities and is a hazardous contributor to the global warming phenomenon [48, 49].

Methane mitigation in ruminants is possible through various strategies. Today, the feeding management approach is the most developed [50], being the basic objective to improve the efficiency of both animal productivity and ruminal fermentation. Among such approaches, are the manipulation of rumen fermentation pattern by shifting to an increment in propionate at the expense of acetate and diminishing the hydrogen available for methane production is the most developed [50]. Use of polyether ionophores and virginiamycin, has proven to be a suitable way to manipulate rumen fermentation profiles as well as providing a stable rumen environment. The reduction in methane production after ionophores utilization has been well documented [48, 51, 52]. Nonetheless, a methane sparing effect by organic acids [53, 54], enzymes [55, 56] and yeast from *Saccharomyces cerevisiae* [57] has also been reported.

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Nitrogen

While the N cycle in nature are is ecologically and biologically relevant, human-driven activities are increasing the amount of N cycling, hazardously affecting ecosystems around the world [58]. Cattle need protein for maintenance and production. The rumen microbiota also need N for microbial growth during the fermentation process of feeds. When more protein N is supplied than the needed, it is excreted primarily as urea in the urine. Ammonia in livestock facilities results primarily from the breakdown of urea by the enzyme urease and may affect livestock and human health when exposed within spaces with suboptimal ventilation [59]. Approaches implemented to decrease ammonia emissions imply precision feeding practices to carefully matching formulation of diets to cattle nutritional requirements and thus improve nutrient utilization, in addition, whole farm programs in order to integrate nutrient management across herd, crop, soil, and manure components certainly will contribute to diminish environmental hazards [60]. Nonetheless, the use of antibiotics, particularly the polyether ionophores, monensin and lasalocid; which were two of the first commercially available ionophores; and the are more recently approved performance enhancing antibiotics for cattle: laidlomycin propionate, bambermycin, and virginiamycin, clearly have an important role in helping to maximise N utilisation [35].

The degradation of proteins in the rumen is considerably diminished when ionophores are incorporated in the diet [61]. Early studies have indicated that ionophores have a significant impact on ruminal nitrogen retention, a phenomenon referred to as protein-sparing effect, and numerous studies *in vitro* and *in vivo* have indicated that the presence of monensin reduces ammonia-N production in ruminal fluid. Pioneer studies performed by Van Nevel and Demeyer [62] on the effect of monensin on rumen fermentation indicated that ammonia production was inhibited in the presence of monensin in the incubation medium. Accordingly, Russell and Martin [63] also reported a decrease of more than 50% in ammonia production in studies carried out to determine the effect of methane inhibitors on amino acid fermentation by rumen microorganisms *in vitro*.

The early findings related to the effect of monensin on ammonia production in rumen were difficult to explain. Gram-negative bacteria were considered to produce more ammonia than the majority of the isolated Gram-positive species. However, Russell *et al*. [64] observed that mixed cultures produced more ammonia than pure cultures of Gram-negative bacteria. These results led to Russell and co-workers to hypothesize that very active ammonia-producing bacteria might have not yet been isolated. They, indeed, were able to isolate two Gram-positive bacteria, a *Peptostreptococcus* and a *Clostridium*. Both isolates had much higher specific activities of ammonia production much higher than rumen bacteria previously examined [64, 65]. These bacteria are considered obligate amino acid fermenters and their sole function is deamination of amino acids. Therefore, the protein sparing effect of monensin may be related in part to the inhibition of the Gram-positive bacteria isolated by Russell and co-workers, which presented a high deaminative activity.

However, Wallace [66] indicated that, although the decrease in ammonia production observed when ionophores are incorporated in the diet is primarily due to a partial elimination of high deaminative bacteria, the effect of antibiotics on the deaminative activity of insensitive species should be taken into consideration.

ANTIMICROBIALS FOR DISEASE CONTROL

Antibiotic use in food animal production is a necessary and integral part of assuring an abundant, wholesome supply of food, protecting animal health and relieving animal suffering. Antibiotics can be used at continuous low levels for improvements in rate of gain and efficiency. Nonetheless, higher levels of antibiotics typically are needed for therapeutic, metaphylactic and prophylactic approaches of diseases or conditions such as scours, bacterial enteritis, coccidiosis, shipping fever, anaplasmosis, foot rot, among some others. Chlorotetracycline, oxytetracycline, bacitracin, neomycin, cephlosporins, are examples of antibiotics intended for specific disease prevention or treatment [67].

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RISKS RELATED TO THE USE OF ANTIBIOTICS IN ANIMALS

Modern livestock production systems use antibiotics and other antimicrobials to prevent or to treat disease in animals. In addition, the use of antibiotics to improve growth and feed efficiency has been performed since the 1950s. This is a common practice in livestock feeding systems through the use of continuous, low-dose administration. A continual, non-therapeutic use of drugs is sometimes performed for long periods of time, or even for the whole life of the animal, thus leading to the development of antibiotic resistance, particularly in bacteria from the genera *Enterococci* [68].

Antibiotic resistance limits treatment options, delays recovery and costs more. The most troubling aspect is that antibiotic resistance can increase with continued and widespread use of antibiotics to improve growth [69].

In addition, indiscriminate use of antibiotics may result in selection of antibiotic-resistant bacteria. Resistance can negatively impact both animal and human health. Single or miltimulti-drug resistance can occur. Some of these resistant bacteria can directly cause disease in livestock, or they can pass their resistance traits to disease-causing bacteria. Humans can acquire these resistant bacteria either through direct contact with infected animals or through contaminated food or water. Thus, the use of animal drugs, antibiotics in particular, is currently capturing a world wide public attention which focuses primarily on the favorable and unfavorable effects of animal drug use on human health. Antibiotic agents are one class of drugs used extensively in food-animal production and the most important concerns among population today are microbial resistance to these compounds and residues of these compounds in the food supply [20].

Additional concerns are driven by the fact that antibiotics that come from both animal and human uses are making their way into rivers and streams [70].

It represents a relevant issue because such compounds may become a hazard for the ecosystem and to humans through drinking water as well [71].

In current agricultural practice, raising animals for food depends heavily on the use of pharmacologically active compounds, which is fundamental to animal health and well-being and to the economics of the industry. The gains that have been made in animal food production capacity would not have been possible without the ability of reliable chemicals to contain the threat of disease to animals [20]. Nonetheless, any use of antibiotics in animal or human medicine increases the risk of emergence of resistant bacteria, and unfortunately, the

greatest risk occurs with irresponsible overuse of one antimicrobial. Further efforts must be aimed at evaluating alternative means to manipulate the gastrointestinal microflora in production livestock if progress is to be made in reducing hazardous risks for human health [72]. Finally, it is fundamental to reach effective collaborative networks among government officials and industry to promote a prudent use of antibiotics in livestock.

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CONCLUSION

Antimicrobial drugs are commonly used in food-producing animals to prevent and control disease, as well as to improve growth and feed conversion efficiency. Many of the conventional diets used for feeding livestock contain one or a combination of several antibiotics, as well as other antimicrobial growth promoters. These additives help farmers to overcome significant reductions of daily weight gain and feed efficiency, but this largely depend on the actual hygienic status in livestock operations. However, there have been concerns about the safety of using subtherapeutic antibiotics to promote weight gain, such as drug resistance in several important human pathogens. The economic impact of banning antibiotics is huge, with consumers likely sharing most of it. The collateral consequences of feeding subtherapeutic doses of antibiotics to food-producing animals must be taken into account in cost-benefit analyses.

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Chapter 3

ASPARAGOUS RACEMESUS AS NATURAL FEED ADDITIVE APPLICATION FOR RUMINANTS AND NON-RUMINANTS HEALTHY PRODUCTS

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ABSTRACT

The use of herbal feed additives in livestock production is as old as the ancient history. This chapter enumerates traditional ethno-veterinary knowledge about *Asparagus racemosus.* The emphasis has been laid on the scientific validation of the medicinal properties of this plant that is used in traditional medication system of domestic animals, and to know and reward the traditional wisdom of the local communities. There have been very few studies on the ethnobotanical studies of this plant that are used for the treatment of livestock diseases. This study aims to document knowledge about ethnobotanical information of Shatavari used for the treatment of livestock diseases.

Keywords: Ethno veterinary, Medicinal plants, *Asparagus racemosus*, Traditional knowledge

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INTRODUCTION

The use of herbal feed additives in livestock production is as old as the ancient history. Nowadays, herbal plants are widely used as animal feed additives [1, 2]. Traditionally, *Asparagus racemosus* (Shatavari) commonly known as "queen of herbs" has been used to treat neurodegenerative disorders such as senile dementia associated with Alzheimer's disease [3, 4]. *Asparagus racemosus* Willd. (family Asparagaceae; Liliaceae) is commonly called Satavari, Satawar or Satmuli in Hindi; Satavari in Sanskrit; Shatamuli in Bengali; Shatavari or Shatmuli in Marathi; Satawari in Gujarati; Toala-gaddalu or Pilli-gaddalu in Telegu; Shimaishadavari or Inli-chedi in Tamil; Chatavali in Malayalam; Majjigegadde or Aheruballi in Kannada; Kairuwa in Kumaon; Narbodh or Satmooli in Madhya Pradesh; and Norkanto or Satawar in Rajasthan [5]. The Asparagus genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant [6]. Shatavari act as antiseptic, astringent diuretic and galactogogue properties. Roots contain saccharine and mucilaginous substances in large proportion. It helps in improving digestibility, increase milk after calving, helps for mammary gland development, hastens letting down time, stimulate lactiferous tissues and keeps udder and treats in smooth condition [7].

The majority of these natural products are produced from the fermentation broth of microorganisms, though plants have also been an important bioactive source. There is increasing public concern regarding the use of pharmaceuticals in the animal industry. Much of this has been as a result of the emergence of drug resistance. A particular area of criticism has been in the use of antibiotics as growth promoters and the associated risk of developing antibiotic resistance in human pathogens [8]. Roots of *Asparagus racemosus* (Shatavari) are used in various medicinal preparations. This herb is traditionally used to rectify the gynecological problems and sexual dysfunction [9].

It also supports deeper tissue and builds blood and thus helps to remove infertility, prepare the womb for conception, prevents miscarriage and acts as a postpartum tonic where it helps to increase lactation and normalize the uterus after parturition, prolapse of the uterus and the changing hormones [10].

In Ayurveda this amazing herb is called the "queen of herbs" as it increases the capacity for milk production and side by side improves the vitality of fetus and newly born. Therefore, its supplementation is recommended during last trimester of pregnancy to the first trimester of post parturition to the mother to boost milk quality and immunity of both mother and fetus [11]. The scientific evidence of galactopoietic and mammogenic properties of *A. racemosus* are reported in dairy animals with varying doses, without considering the body weight of supplemented animals thus, dissimilarity of dose/day/kg body weight of the animal in a group [12, 13].

Shatavari has more than 50 organic chemical compounds of different groups such as steroidal saponins, glycosides, alkaloids, polysaccharides, mucilage, racemosol and isoflavones that possess a wide range of medicinal properties [14].

The literature available on shatavari (*Asparagus racemosus*) supplementation and its effect on digestion, milk production, udder and reproduction health, energy metabolism, immune system and blood metabolites of dairy cows has been reviewed as per the objectives of the study under the following heads:

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EFFECT OF SHATAVARI ON ANIMAL PERFORMANCE

1. Milk Production

The general pharmacology of shatavari are galactagogue and mammogenic, it enhances the blood prolactin level and stimulates the cellular division of mammary glands [15, 16, 17]. The galactopoietic effect of satawar (shatavari) has been reported since long ago and it has been reported that the supplementation of fresh root of shatavari concentrate at the rate of 0.5 kg per day, at the time of milking, increases the milk yield of buffaloes significantly ($P<0.01$) [18]. Similar results have also been reported in the case of freshly calved crossbred cattle that oral supplementation of root powder of satawar at the rate of 100 g per animal, on alternate days, improved milk production significantly ($P<0.05$). The glucogenic property of *Asparagus racemosus* supplementation in dairy cows was also reported [19]. The glucogenic property of *Asparagus racemosus* in cows might be due to the presence of saponin. Saponin in *Asparagus racemosus* might have increased the propionate production, which has generally been considered the major substrate for gluconeogenesis [20], thereby resulting in improved blood glucose levels. Alexander [21] reported that saponin isolated from the glucogenic property of *Asparagus adscendens* roots aqueous extract, which is similar to *Asparagus racemosus*, enhanced the production of propionate in growing sheep. Similarly, Abreu *et al*. [22] and Hu *et al*. [23] also reported that supplementation of saponin in the diet improved the ruminal volatile fatty acids (VFA) profile, microbial efficiency and propionate significantly. A significant increase in milk yield has also been observed in guinea pigs and goats after feeding lactare which also increased growth of the mammary glands, alveolar tissues and acini in guinea pigs [24]. Extract of shatavari has been shown to increase both the weight of mammary lobulo-aveolar tissue and the milk yield. This effect was attributed to the action of released corticosteroids or an increase in prolactin [25]. Shatavari has been found to stimulate milk production in buffaloes [18]. The galactogenic effect has been confirmed by a clinical trial [26]. Lohar *et al*. [27] demonstrated that *Asparagus racemosus* roots is a rich source of minerals and trace elements such as calcium, copper, magnesium, iron, manganese, nickel and zinc. The presence of calcium in the powder correlates with the analytical results for galactogogues. Baig and Bagwat [28] explained that Galactin Vet bolus (contains powders of *Leptadenia reticulata, Asparagus racemosus, Withania somnifera, Arundo donax, Cissampelos pareira, Foeniculum vulgare*) improved milk yield, Fat %, SNF % and corrected lactometer reading (CLR) in dairy cows. There is no adverse effect of Galactin N Vet bolus on the composition of milk. Also, there were no adverse effects on health of animals during the study period. Hence, Galactin Vet bolus can be used safely as galactogogue in dairy animals. Mayo [29] and Rao [30] reported that the root powder obtained after extraction with chloroform and methanol (1:1) was added in different percentages to the animal feed. Rats fed on a 2 % *Asparagus racemosus* diet showed a significant ($P<0.05$) decline in both tumor incidence and mean number of tumors per tumor bearing animal. They concluded that *Asparagus racemosus* root extract exerted a mammotropic and/or lactogenic influence on normal as well as on oestrogenprimed animals thereby rendering the mammary epithelium refractory to the carcinogen. Sabnis *et al*. [16] found that the aqueous extract of *Asparagus racemosus* roots increased the weight of mammary glands in post-partum and oestrogen-primed rats and the uterine weight in the oestrogen-primed group. This effect could be

attributed to the action of released corticoids or prolactin. Oral administration of the alcoholic extract of *Asparagus racemosus* rhizome (30 mg/100 g body weight, daily for 15 days) to pregnant female adult albino rats had an oestrogenic effect on the female mammary glands and genital organs [17].

The administration of alcoholic extract of *Asparagus racemosus* rhizome in pregnant female adult albino rats suggests an estrogenic effect of shatavari on the female's mammary gland and genital organs [31]. The extract of shatavari has been shown to increase both the weight of mammary lobulo-alveolar tissue and the milk yield in animal experiments. This effect was attributed to the action of released corticosteroids or an increase in prolactin. Shatavarins I-V, the steroidal saponins, may be responsible for the hormonal like effect of shatavari and explain its traditional use as a reproductive tonic [17, 32]. The presence of steroidal saponins and sapogenin constituents has been shown to directly contribute in the lactogenic effect of *Asparagus racemosus* [33, 34]. Its galactogogue activity was evaluated experimentally on rats, cows, buffaloes and goats [18, 35]. Kumar *et al*. [36] evaluated the effect of shatavari supplementation on milk production and its composition. Our results indicated that supplementation of shatavari root powder prepartum (60 days) at the rate of 100 mg per kg live body weight, continuing postpartum period (90 days) at the rate of 200 mg per kg live body weight, improved milk production and its composition. Besides improving the milk fat content it reduced milk fat (ghee) total cholesterol and increased net return per liter of milk significantly in crossbred cows. Therefore, it is concluded that shatavari supplementation is economically viable and beneficial, and it could serve as a potential management tool to improve milk production, composition and net returns from lactating crossbred cows.

2. Reproduction

Asparagus. racemosus has rejuvenative properties and it stimulates the epithelial cell division resulting in early healing of the uterine wall and leads to early uterine involution and consequently an early initiation of the estrus cycle in supplemented groups [17, 37]. The improvement in supplemented group could be due to the anti-oxytotic action of the *A. racemosus* compound present in *A. racemosus* on the uterus, which helps in conception [32]. Mitra et al. [38] also reported that a *A. racemosus* based herbal formulation did not possess oxytocin like activity which might be useful in conditions associated with hypermotility of the uterus as in threatened abortion, hence *A.racemosus* supplementation enhances conception. It has been indicated as a uterine tonic, thus it cleanses, nourishes, and strengthens the female reproductive system and so is traditionally used for premenstrual syndrome and sexual debility [37], ammenorrhea, dysmenorrhea, dysfunctional uterine bleeding [37, 39, 40], menopause and pelvic inflammatory disease like endometriosis [41, 42], and gonorrhea [14]. It also supports deeper tissue and builds blood and so it helps to remove infertility, prepare the womb for conception, prevents miscarriage and acts as a post-partum tonic where it helps to increase lactation and normalize the uterus, prolapse of uterus and the balancing reproductive hormone levels [10]. Other traditional uses are in arthritis [43], diarrhea, dysentery [44], edema, rheumatism, chronic and common fevers, aphrodisiac, cooling tonic, antispasmodic [45, 46], dyspepsia, indigestion [47], thirst, sunstroke [48],

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rejuvenator, promoter of strength, breast milk and semen [49], long-term treatment of diabetes, cough [50], and peptic ulcers [51, 52]. Shatavari is also used for enhancing milk production in the freshly parturient and lactating woman [39]. In spite of cholinergic activity of *A. racemosus* on guinea pigs ileum, ethyl acetate and acetone extracts of the root of *A. racemosus* blocked spontaneous motility of the virgin rat's uterus. These extracts also inhibited contraction, induced by spasmogens like acetylcholine, barium chloride and 5-hydroxytryptamine whereas alcoholic extract was found to produce a specific block of pitocin induced contractions.

On the other hand, petroleum ether as well as ether extracts of the powdered roots did not produce any uterine activity. It indicates the presence of some particular substance in the alcoholic extract which specifically blocks pitocin sensitive receptors though not other receptors in the uterus [15], confirming that shatavari can be used as uterine sedative. Further, a glycoside, shatavarin I, isolated from the root of *A. racemosus* has been found to be responsible for the competitive block of oxytocin-induced contraction of rat, guinea pig and rabbit's uteri, *in vitro* as well as *in vivo* [36, 53] studied on ten crossbred cows which were classified in 2 groups of 5 each, viz. C (control) and prepartum (i.e. 50 to 60 days dry period) to continued postpartum period (up to 90 days postpartum).

During prepartum, the cows in group prepartum to postpartum were supplemented with *Asparagus racemosus* root powder at the rate of 100 mg/kg live body weight and it was continued during postpartum period at the rate of 200 mg/kg live body weight. The supplementation of *A. racemosus* during both prepartum continued to postpartum resulted in significant, improvement in per day milk production and reduction in first postpartum oestrus interval, service period (day) and services per conception, and rate of uterine involution in comparison to the control group. Roots of *Asparagus racemosus* (shatavari) are used in various medicinal preparations.

This herb is traditionally used to rectify gynecological problems and sexual dysfunction [54]. It also supports deeper tissue and builds blood and thus helps to remove infertility, prepare the womb for conception, prevents miscarriage and acts as a postpartum tonic where it helps to increase lactation and normalize the uterus after parturition, prolapse of uterus and the changing hormones [10]. The *A. racemosus* has rejuvenative properties and it stimulates the epithelial cell division resulting in early healing of uterine walls and leads to early uterine involution and consequently early initiation of the estrus cycle in supplemented groups [17, 35, 37].

It was reported that administration of a *A. racemosus* based herbal formulation increases the thyroxin stimulating hormone, follicular stimulating hormone and luteinizing hormone, which helps in regular ovulation and improved conception rate significantly ($P<0.05$) in infertile women.

It was reported that aqueous extract of *A. racemosus* root significantly ($P<0.05$) reduced the excretion of calcium and phosphorus in the urine and thus enhanced the resulted availability, a significant improvement in the mineralization of bone [55, 56]. It was reported that supplementation of polyherbal immunomodulator containing *A. racemosus* at the rate of 250 mg/kg body weight during both pre and postpartum period in crossbred cows resulted in a decrease in mastitis incidence and a significant increase in daily milk yield.

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3. Immunomodulatory Activity

Immunomodulation is the regulation and modulation of immunity either by enhancing or by reducing the immune response. Modulation of immune response may involve induction, expression or amplification of the immune response [57, 58]. Studies on the haematobiochemical parameters of a feed diet supplemented with different levels of shatavari root powder (SRP) in broiler chicks, there is a significant ($P<0.01$) variation for herb, total serum protein, albumin and globulin between treatments. As the level of SRP increased in the diet, an increasing trend was observed which might support the immune system of birds. Gautam *et al*. [59] showed that the treated animals showed a significant increase in antibody titers as compared to untreated animals after challenge ($P=0.002$). Immunoprotection against intra-cerebral challenge of live *Bordetella pertussis* cells was evaluated based on degrees of sickness, paralysis and subsequent death. Reduced mortality accompanied with overall improved health status was observed in treated animals after intra-cerebral challenge of *Bordetella pertussis* indicating development of a protective immune response. The present study indicates applications of test materials as a potential immunoadjuvant that also offers direct therapeutic benefits resulting in less morbidity and mortality. Rege *et al*. [60] administered orally the aqueous, standardized extract of *Asparagus racemosus* to experimental animals, following which they were exposed to a variety of biological, physical and chemical stresses. Using a model of cisplatin induced alterations in gastrointestinal motility; the ability of this extract to exert a normalizing effect, irrespective of direction of pathological change was tested. *Asparagus racemosus* reversed the effects of cisplatin on gastric emptying, and also normalized cisplatin induced intestinal hypermotility. Bhattacharya *et al*. [61] undertook a study to investigate the adaptogenic activity of 'Siotone' (a herbal formulation consisting of *Withania somnifera*, *Ocimum sanctum*, *Asparagus racemosus*, *Tribulus terristris* and shilajit) against chronic unpredictable, but mild, foot shock stress induced perturbations in behavior (depression), glucose metabolism, suppressed male sexual behavior, immunosuppression and cognitive dysfunction in albino rats. The stress indices for evaluation were gastric ulceration, adrenal gland and spleen weights, ascorbic acid and corticosterone concentrations of adrenal cortex and plasma corticosterone levels. Root powder of *Panax ginseng*, a reputed 'rasayana' herb was used as the standard adaptogenic agent for comparison purposes.

Gupta *et al*. [62] studied twenty growing crossbred heifers, 6 to 12-months old and weighing 59 to 96 kg, which were selected and divided into 3 groups of 7 animals each in such a way that the average body weights in all the groups remained uniform. All the experimental animals in different groups were fed similar basal rations (group 1, control) whereas, animals in group 2 were supplemented with a mixture of *Asparagus racemosus* and *Cryptolepis buch* (lnani herbs) and animals in group 3 were supplemented with a mixture of *Bacapa monnieri, Urtica dioica* and *Eclipta alba* herbs at a 1% level of concentrate mixture as feed additive. Hematological parameters, viz. haemoglobin (Bb), packed cell volume (PCV), total leukocyte count (TLC) and differential leukocyte count (DLC); serum total protein, albumin, globulin and albumin: globulin ratio, urea nitrogen and activities of AP, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SOPT) and blood glucose level did not differ significantly among the different treatment groups. It was concluded that the inclusion of herbs at 1% level in concentrate mixture as a

feed additive posed no stress and had no harmful effect on crossbred heifers as assessed from haemato-biochemical constituents. Diwanay *et al.* [63] reported that both *W. somnifera* and *A. racemosus* extracts stimulate immune response activities in immunosuppressed busectomized and thymectomized broiler chicks supplemented with DABG and DATG respectively. Extracts of *W. somnifera* and *A. racemosus* also showed immunopotentiating effects in a cyclophasphamide treated mouse with ascetic sarcoma. Kumari *et al*. [64] showed that the use of *W. somnifera* and *A. racemosus* dried root powder in a specific dose during the scheduled period showed significant positive effects on both humoral and cell mediated immune responses of the birds. The bursectomized and thymectomized birds showed a decline in the antibody titer. The variation in skin thickness was significantly more among those herbally treated. Herbal formulations containing extracts of *W. somnifera* and *A. racemosus* may therefore be recommended for use as a positive immunomodulator in normal and immunocompromized broiler chicks. The present study also indicated the determinative roles of herbal feed additives in helping the immunodeficient subjects in obtaining higher humoral and cell mediated immune responses, providing better protection level against infections.

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4. Antioxidant and Antinflammatory Activities

Recent reports on *Asparagus racemosus* indicate that the root extracts have antioxidant and antidiarrheal activities in laboratory animals [65, 66, 67]. Studies investigated the potential of *Asparagus* root (powder) as a dietary supplement in normalizing the hyperlipidemic/ hypercholesteremic and oxidative stress conditions induced by chronic intake of lipid-rich diets with regard to plasma and hepatic lipid profiles, cholesterol metabolism and antioxidant status. Additionally, the effect of *Asparagus* root was assessed on normocholesteremic animals with a view to investigate and to compare its influence on lipid metabolism in both normal and hyperlipidemic rats. The lipid-lowering effects of *Asparagus racemosus* root in hypercholesteremic rats demonstrated in the present investigation are related primarily to an increased excretion of cholesterol, neutral sterols, bile acid and an increase in hepatic bile acid content. Asmari *et al*. [68] determined the pharmacological (Anti-inflammatory) potential of *in vitro* cultures of *Asparagus racemosus*. Anti-inflammatory activity of methanolic extracts of shoot, root their calli and genetically transformed shoot culture was performed using a carrageenan induced rat paw edema method. The albino rats were treated orally with two dosages (500 or 1000 mg/kg body weight of rats) of drugs, one hour prior to injection of carrageenan (0.1 ml, 1%, intraperitoneally) in the right hind paw. The paw volume was measured just before and up to 3 hours after the injection of carrageenan. Methanolic extract of root (1000 mg/kg body weight) showed the best result (81.81% inhibition) followed by root callus extract. The natural root extract showed significant anti-inflammatory activity whereas in cultured cells only root callus extract (500 mg/kg body weight) was found to give better result. Rekhate *et al*. [58] worked on an eighty day old straight run of commercial broilers chicks, which were randomly distributed into four groups (T_1, T_2, T_3 and T_4) of two replications having ten chicks in each, reared on a deep litter system up to 8 weeks of age. The chicks in all the treatment groups were fed basal diet T_1 control and T_2, T_3 and T_4 groups were fed basal diet + 0.5, 1.00 and 1.5 per cent SRP respectively. A significant ($P<0.01$) improvement in the live body weight of broilers was

observed between the treatments. Better feed conversion efficiency was observed with the increase in SRP. The digestibility of protein was significantly ($P< 0.01$) better with a higher inclusion level of SRP. Hemoglobin, total serum protein, albumin and globulin revealed significant ($P<0.01$) variations between the treatments. It was concluded that supplementation of SRP at 0.5, 1.00 and 1.5% levels improves growth performance and general health status of broilers however, a 1.0% level could be more economical.

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CONCLUSION

This chapter evaluates the biological activities, pharmacological applications and clinical studies of *Asparagus racemosus* in an attempt to provide a direction for future research. The World Health Organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs to cure their disease. *Asparagus racemosus Willd*. or Shatavari is a creeper of the plant genus *Asparagus*. Its medicinal properties are reported in traditional systems of medicine such as Ayurveda, Siddha and Unani. Only a few studies have been done on dairy animals of this herb which are of pharmacological and chemical type. However, there is a growing human awareness and demand for chemical residue-free and clean products such as milk and meat. Thus, there is a need to carry out more bio-chemical work and detailed studies on dairy and meat animals. Utilization of this herb will not only improve the reproductive efficiency and health of domestic animals but also support the farmer's income through organic production of more milk and meat per animal.

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Chapter 4

TROPICAL TREE FOLIAGE AND SHRUBS AS FEED ADDITIVES IN RUMINANT RATIONS

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ABSTRACT

In the tropical regions of Mexico there are available many types of foliage and pods of trees and shrubs which hold potential to be used for ruminant feeding during critical periods of the year. This vegetation usually contains good concentrations of crude protein and fermentable carbohydrates but some are only seasonally available or may contain different types of secondary metabolites with potentially deleterious effects on animal health and productivity. This chapter presents some of the data obtained in this laboratory concerning the effect of the addition of several levels of foliage on intake, digestibility and productive performance of tropical sheep. When high quality foliage such as *Brosimum alicastrum* and *Leucaena leucocephala* are consumed by ruminants, they usually increase ammonia nitrogen concentration in the rumen and thus microbial protein synthesis in this organ. Dry matter intake was generally increased when highly fermentable foliage were added to the ration of sheep, increasing passage rate from the rumen. Types of tropical legumes such as pods of *Enterolobium cyclocarpum* and *Acacia pennatula* were added to the rations of growing sheep at various levels and the daily weight gain recorded was comparable to that obtained with concentrate rations, although feed conversion efficiency was lower. It is concluded that foliage and pods of tropical trees and shrubs have ample potential to improve ruminant production in the tropics,

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being able to even improve the quality of meat and milk by increasing the concentration of unsaturated fatty acids (conjugated linoleic acid or CLA) in ruminant products.

Keywords: Foliage, pods, sheep, nutritive value, rumen, tropics

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INTRODUCTION

Feeding ruminants in the tropics is based on the use of native and introduced grasses, with biomass production being limited by seasonality of rainfall. This is reflected in low nutritive value and digestibility [1, 2]. Under those conditions, productive performance of ruminants is low and many producers rely on the use of increasing amounts of starchy feedstuffs [3] as the base of the feeding of their animals. However, the cost of concentrates has been increasing during the last few years resulting in lower profitability for the farm owner.

The use of local feed resources is therefore an option for small and medium size producers to reduce production costs and increase profitability [4]. In the tropical regions there are foliage trees and shrubs of good nutritive value with potential to be incorporated in the ration of growing ruminants as a partial substitute of grains in integral rations [5]. During the dry season (April-May), pods of *Acacia pennatula* and *Enterolobium cyclocarpum* become available. A yield of 86 kg of fresh pods of *E. cyclocarpum* per tree has been reported [6]. In an experiment carried out in Honduras with hair sheep, researchers [7] were able to incorporate up to 36% of ration dry matter with ground pods of *E. cyclocarpum* reporting a daily weight gain of 223g/lamb. Another option in the attempt to optimize animal response is the use of foliage of many trees and shrubs locally available as additives in ruminant feeding. Plant diversity within the paddocks provides several kinds and qualities of foliage, legumes and wild plants which allow the animal to balance its diet so as to cover their nutrient requirements [8]. Feeding proposals are directed towards the incorporation of locally available resources of Southern Mexico in rations for ruminants. Foliage and pods of plants such as *Leucaena leucocephala, Guazuma ulmifolia, Samanea saman, Brosimum alicastrum, Acacia pennatula, Enterolobium cyclocarpum, Mucuna pruriens,Canavalia ensiformis, Gliricidia sepium*, have demonstrated their potential to be incorporated in various proportions in practical rations [9].

FORAGE TREES AS FEED ADDITIVES

Forage trees contribute towards sustainability of agricultural systems by increasing nutrient cycling, erosion control, improving the physical and biological conditions of the soil and as elements for reforestation of agroecosystems [10]. For a tree to be considered as a source of forage, it must have appropriate nutritional quality: its consumption must promote positive changes in productive parameters, secondary metabolites should not affect feed intake and the plant must resist cutting and maintain good yields of biomass. In this sense, due to the fact that the diversity of forage trees in the tropics of Mexico have a relatively high content of crude protein in their foliage [11, 12] it is appropriate to consider them as protein

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additives for grazing ruminants during the dry season or to be used when agricultural byproducts (straws) which contain low amounts of crude protein are fed. It is important to point out that the basic requirements to maintain an appropriate intake of any foliage is to maintain an adequate rumen environment, optimum for the development and activity of cellulose degrading bacteria in the rumen. Thus, a rumen pH among 6.3 and 7.0 is required, with ammonia (NH_3) concentration above 5 mg 100/ml for the maximal growth of rumen microbes [13]. In addition, a good digestibility value is necessary to keep food intake high. In this context, it has been observed that when cattle are supplemented with tree foliage, it is possible to improve digestibility of the grasses (low in crude protein). Tree foliage in the ration of ruminants usually increase NH_3 concentration in the rumen above minimum values and maintain a rumen pH adequate for the growth of the fiber degrading bacteria [14], it is in this way that the positive effect on feed intake and animal productivity is reached [12]. In Mexico, there is a large amount of trees and shrubs which are utilized as sources of food for domestic animals in the arid, semi-arid and tropical regions, among which are notorious legumes such as *Acacia spp*., *Albizia spp*., *Leucaena spp*., *Eysenhardtia polystachya*, *Gliricidia sepium*, *Erythrina spp*., *Prosopis juliflora*, *P. laevigata*, *Calliandra spp*., *Pithecelobium spp*. and *Caesalpinia spp.* among others. It has been pointed out [9, 15] that in Mexico there is a large variety of plants with the potential to be incorporated in ruminant production systems, which could lead to more sustainable livestock systems by making them less dependent on external inputs (protein and energy additives) which have a high cost at present.

NUTRITIVE VALUE OF TREE FORAGES

The use of foliage, seeds and pods of trees and shrubs in ruminant feeding has been a known practice for many years; however at present, the integral management of resources and the knowledge of the nutritional characteristics, allows the utilization of a diversity of species as feed additives for ruminants with the aim of improving productive parameters (weight gain, milk production) in production systems. The nutritive value of pods is based on the concentration of soluble sugars, crude protein as well as other nutritive components (Table 1).

In addition to the high nutritive quality of pods, these have a relatively good yield, for example it has been determined that *Enterolobium cyclocarpum* may produce up to 86 kg of fresh pods per tree [6]. On the other hand, due to the chemical composition of foliage trees, these could improve the productivity of grazing ruminants basically because the available forage is of low quality particularly during the dry season [16]. *Brosimum alicastrum Swartz.* is a widely available moraceous tree present in some areas of the tropical and subtropical regions. It provides seeds and foliage for the feeding of cattle, pigs, goats, sheep and horses [18]. A tree of *B. alicastrum Sw*. may produce up to 57 kg of dry foliage per year. Dry matter content of the leaves is 50% and of the branches with diameter among 0.5 to 1.5 cm of 56.6%. The potential of the foliage for the feeding of ruminants is based on the crude protein content, which is enough for the maintenance of the rumen microbes (>7%; [19]). Voluntary intake of foliage of *B. alicastrum* varies among 4.0 and 6.0 kg DM/100 kg live weight in cattle [20]. Chemical composition of the foliage of *B. alicastrum* is shown in Table 2.

Table 1. Chemical composition (g/kg DM) of foliage of tropical trees

Species	CP	NDF	ADF	TP	CT
Acacia farnesiana	240	421	267	10	45
Acacia milleriana	118	427	285	35	73
Acacia pennatula	125	590	358	28	39
Albizzia caribaea	166	367	310	9	0
Bauhinia ungulate	132	424	265	42	0
Diphysa robinioides	187	317	232	6	14.9
Erythrina goldmanii	228	431	288	6	1.5
Genipa americana	94	377	309	9	2.7
Gliricidia sepium	238	385	247	3	0
Guazuma ulmifolia	104	425	295	28	47.1
Leucaena leucocephala	201	275	191	3	128
Phitecollobium dulce	196	452	293	6	0

CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; TP: Total phenols; CT: Condensed tannins [17].

Table 2. Chemical composition of foliage of *Brosimun alicastrum Sw* [21]

Item	(%)
Dry matter	88.7
Crude protein	14.2
NDF	37.5
ADF	26.0
Cellulose	19.6
Hemicellulose	11.4
Lignin	6.5
Gross energy (calorie/g)	3830

In an experiment carried out in Mexico [14] with rumen cannulated Pelibuey sheep housed in metabolic cages and fed hay of *B. alicastrum* as an additive at levels of 0 (control), 15, 30 and 45% of ration dry matter in a basal ration of *Panicum maximum* added with 12% of cane molasses, it was found that voluntary intake was increased as the level of *B. alicastrum* was increased. Sheep without foliage of *B. alicastrum* in their ration (control), consumed significantly less dry matter than the other animals (Table 3). Similar results have been recorded with other species. With cattle, an increase was observed [22] in total DM intake from 3.39 to 4.45 kg/day in Cebu x Brown Swiss bulls, when the basal ration of sisal pulp (*Agave fourcroydes*) was added with fresh foliage of *B. alicastrum* at 2% of live weight. Similarly, it has also been demonstrated [23] an increase in intake of the basal ration of Pelibuey sheep when the ration was added with 30% of the DM as foliage of *B. alicastrum*. Generally speaking, voluntary intake of DM and OM of ruminants are increased when poor-quality forages are supplemented with protein foliage from trees and shrubs. With Pelibuey sheep fed a basal ration of *Cynodon nlemfuensis* added with 30% of ration dry matter as dry foliage of *Gliricidia sepium*, it has been reported [24] a DM intake of 71.5 g/kg$^{0.75}$, a value similar to that of 70.2 g/kg$^{0.75}$obtained when foliage of *B. alicastrum* was added to a sheep

ration [14]. Such similarity in the data obtained may be due to the good crude protein content and rumen degradability of both types of foliage which supply fermentable N to the rumen microbes. The linear increase in intake of DM, OM, NDF and ME (MJ/day) when foliage are added in a ration is probably due to the increase in the rate of passage of particulate matter (k_1) through the rumen, which is likely to improve efficiency of microbial protein synthesis in this organ. It was reported [10] in Pelibuey sheep fed low-quality basal forage rations added with five mixtures of *Brosimum alicastrum* and *Lysiloma latisiliquum* in the proportions: 100:0, 75:25, 50:50, 25:75 and 0:100, that DM intakes were highly significant for the mixtures relative to a control treatment of hay only (Table 4). This agrees with other authors [25] who mentioned that feed intake expressed as g/kg$^{0.75}$/day ranges from 30 g/kg$^{0.75}$/day for mature forage, up to 140 g/kg$^{0.75}$/day, for immature temperate pastures. It is known that intake of tropical forages in ruminants may be affected by the characteristics related to the animal, the feed or the environment. It has been emphasized [26] that voluntary intake of tropical grasses may be regulated by the concentration of fiber, physical fill and digesta load in the rumen, along with its chemical composition, which may be related to high values of lignin (155 g/kg DM) found in *L. latisiliquum*, which determined the low DM intake.

Table 3. Voluntary feed intake of Pelibuey sheep fed a basal ration of *Panicum maximun* added with increasing levels of foliage of *Brosimum alicastrum Sw* [14]

	Level of incorporation of foliage of *B. alicastrum* % DM			
Intake	0	15	30	45
DM g/day	511	848	1106	1313
DM g/kg$^{0.75}$	35	55	70	84
OM g/day	464	758	1032	1191
NDF g/day	364	552	675	771
ME MJ/day	2.6	5.4	8.3	9.8
k_1(%/h)	1.5	2.8	2.8	4.1
Microbial N supply to the				
small intestine g N/ day	2.2	4.9	7.9	9.7

Table 4. Voluntary intake, apparent digestibility and N balance in Pelibuey sheep fed grass hay added with mixtures of *Brosimum alicastrum* and *Lysiloma latisiliquum* at 30 % of total ration[10]

	Brosimun alicastrum:*Lysiloma latisiliquum*					
	100:0	75:25	50:50	25:75	0:100	Control
DM intake g/sheep/d	1059.7	1022.1	999.7	934.2	786.4	869.8
DM intake g/kg$^{0.75}$/d	98.4	94.1	94.0	86.2	72.8	81.9
ME intake MJ/sheep/d	7.7	7.1	6.8	6.3	5.2	5.9
Digestible DM intake	514.5	473.9	458.4	416.4	346.1	394.5
DM digestibility (%)	48.6	46.5	45.7	44.8	44.5	45.2
Intake N g/sheep/d	13.7	12.4	12.7	11.8	9.7	10.0
N excreted in feces g/sheep/d	8.1	8.2	8.9	9.1	7.9	5.4
N excreted in urine g/sheep/d	2.9	2.5	2.1	1.8	1.7	3.3
N retained g/sheep/d	2.7	1.8	1.7	1.0	0.1	1.3
Urea N in urine g/sheep/d	0.3	0.2	0.2	0.2	0.5	0.3

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According to results obtained in that trial [10], adding increasing levels of the foliage of *L. latisiliquum* in the ration resulted in a linear decrease in feed intake (Table 4). Other workers [27] pointed out that supplementation of low-quality rations may not always result in a positive response as is the case of the work with *L. latisiliquum* [10], where in spite of containing a reasonable amount of crude protein (131 g/kg DM), it was unable to increase feed intake, which could be attributed to its high content of NDF or to the presence of secondary metabolites.

The low digestibility in that work may be due to the content of condensed tannins and to the content of NDF and ADF, which induced a greater resistance to microbial attack by the content of indigestible components (lignin) which limit the availability of energy thus constraining the availability of protein in the lower tract. Apparent digestibility values for the ration with *B. alicastrum* (48.6%) was similar to data by previous authors [14] who found a 46.9% value, while the control ration (grass hay) was superior (45.2%) to those values reported for *Panicum maximum* with a crude protein content of 56 g/kg DM with a value of 35.7% for digestibility [14].

The nitrogenous fraction in *B. alicastrum* demonstrated that it is extensively fermented in the rumen by 24 h, which increase NH_3 concentration in the rumen stimulating microbial protein synthesis. It seems that in rations containing *L. latisiliquum*, protein utilization is low, and this may be determined by the concentration of tannins. It has been considered [28] that plants with tannin concentration of 29 g/kg DM may be rejected by its astringency, therefore feed intake is reduced. The high amount of N excreted in feces and the low excretion in the urine in the sheep fed 100, 75 and 50% as foliage of *L. latisiliquum* [10], may be associated with the presence of condensed tannins and to the greater concentration of lignin in the foliage, which affects nitrogen balance.

This agrees with similar data reported previously [29]. According to other authors [30], some of the protein in legumes have low rumen solubility, thus part of the protein may leave the rumen undigested, but being unavailable in the lower tract, which probably explains why more than 80% of N consumed in the rations with *L. latisiliquum* was excreted in the feces. Nitrogen retention in the rations with foliage of *B. alicastrum* (2.7 g N/sheep/d) are similar to data reported previously [29], who evaluated distillery grains (with 210 g CP/kg DM) without the presence of condensed tannins and similar content of cell walls than the ration with *B. alicastrum*.

When N retention was expressed as a percent of that consumed, values were decreasing as the level of *L. latisiliquum* was increased resulting in a low efficiency of N retention. It is known that the urine is the main route for the excretion of a large part of nitrogenous compounds originated in protein metabolism, in such a way that urea is the main product of N metabolism, so the amount of urea excreted by ruminants, as well as the proportion of urinary N excreted as urea, is highly correlated with the intake of digestible nitrogen.

For the rations with 100% and 75% of foliage of *L. latisiliquum*, the proportion of urea N in the urine was higher than values reported in the literature [31], which suggests that sheep may have even mobilized body protein with an increase in urea N in the urine. However, it is worthwile to consider the ratio of protein:energy since it is associated with the efficiency of feed utilization and feed intake [32].

EXTENSIVE SYSTEMS OF SHEEP PRODUCTION

Extensive systems of sheep production in the tropical areas of South Mexico are based on the grazing of native and introduced grasses such as *Panicum maximum, Cynodon nlemfuensis* and *Brachiaria brizantha*. Breeds of sheep normally present are: Pelibuey and Blackbelly and their crosses with Katahdin and Dorper.

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Under such conditions, daily weight gains are low due to seasonality of grass production and to its low quality (high NDF, low crude protein and low digestibility), in addition to the negative effects of gastrointestinal nematodes and heat.

In this context, there is increasing interest for forage resources which satisfy the nutrient requirements (energy, protein, minerals) of sheep and could improve the profitability of production systems. It is believed that tropical legumes hold the potential to be used in sheep feeding since they generally contain a relatively high content of crude protein and induce a good dry matter intake. Table 5 shows dry matter intake and apparent digestibility by Pelibuey sheep fed increasing levels of foliage (leaves and small stems) of *Leucaena leucocephala* mixed with the grass *Cynodon nlemfuensis.*

Statistically significant differences ($P<0.05$) were observed for dry matter intake, these being higher when *L. leucocephala* was added at 30% of ration dry matter. The same trend was observed when intake data was expressed as g DM/$kg^{0.75}$/day, strongly suggesting the highest intake at 30% of the legume in the ration. These values are higher than those reported by other authors [1] of 60 to 66 g DM/ $kg^{0.75}$/day for intake with sheep, when added with Leucaena among 15 and 60% of ration DM. Dry matter digestibility was slightly superior when *L. leucocephala* was added at 30% of ration DM, however this result was not statistically significant. Intake of ME (9.97 MJ/sheep/d) was significantly higher ($P<0.05$) when Leucaena foliage was given at 30% of the ration DM. Nitrogen excretion in urine showed significant differences ($P<0.05$) among treatments increasing gradually as *L. leucocephala* foliage was augmented in the ration.

It has been pointed out [33] that when crude protein value is in excess of 210 g/kg digestible OM, there may be a loss of N from the GIT which is incurred in an energy cost, which may be reflected in lower animal performance. This occurs apparently when excess amino acids are converted to urea in the liver with the concurrent energetic cost in the urea cycle.

Silvapastoral systems of animal production have been suggested as a more environmentally friendly alternative for ruminant production in the Mexican tropics. The use of tree forage legumes such as *Leucaena leucocephala* in silvoapastoral systems [35] may play an important role since they supply critical nutrients such as protein, minerals and fermentable energy.

However, when this legume is planted at high densities as in some silvopastoral farms in West Mexico, feed intake and animal performance may be affected by the unbalance in the availability of protein:energy in the rumen due to the excess NH_3 arising from protein fermentation in the rumen [33,36].

In a performance trial with Pelibuey sheep grazing at two densities of Leucaena: 35,000 and 55,000 plants per hectare in West Mexico (Table 6), a lower weight gain was found at a high density of the legume [37]. The grass was *Panicum maximum*, var. Tanzania.

The lower weight gain for sheep grazing the paddocks with a high density of Leucaena (55,000 plants per hectare) associated with grasses, may be attributed to a higher intake of Leucaena and therefore of crude protein relative to fermentable energy in the rumen, which led to a low efficiency of microbial protein synthesis in the rumen [36], and also to a greater excretion of N in the urine [38].

It can be speculated that the energy cost of urea synthesis in the liver arising from the NH_3 absorbed from the rumen may have impacted upon weight gain.

Other authors [33] have mentioned that ruminant performance may be impaired when crude protein exceeds 210 g/kg digestible organic matter and to the presence of secondary metabolites such as mimosine, which may affect intake and then weight gain [8], which could probably help to explain the lower productive response at the plant density of 55,000 leucaena plants per hectare.

In a trial [39] carried out with sheep housed in metabolic cages with rations in which 30 and 70% of leucaena was added in a cut and carry system, it was found that when leucaena was above 40% of ration DM there was a reduction in feed intake and weight gain.

This same trend of a reduction in live weight gain of sheep has been reported [1], as the level of Leucaena was increased from 15 to 60%, observing the best weight gains and digestibilities between 30-45% of addition of the foliage.

Table 5. DM intake, apparent DM digestibility, digestible DM intake and urinary N excretion by Pelibuey sheep fed increasing levels of foliage of *L. leucocephala* mixed with *C. nlemfuensis* grass[34]

	Percent incorporation of foliage of *L. leucocephala* in the ration (DM basis)					SEM
	0	15	30	45	60	
DM intake g/sheep/d	950	1010^{ab}	1200^{a}	1115^{ab}	1089^{ab}	119.78
DM intake g/kg$^{0.75}$/d	71^{b}	75^{ab}	89^{a}	82^{ab}	81^{ab}	8.56
DM digestibility %	58^{a}	54^{a}	62^{a}	61^{a}	56^{a}	7.70
Digestible DM intake g/sheep/d	581^{a}	571^{a}	755^{a}	687^{a}	633^{a}	126.78
ME intake MJ/sheep/d	7.60^{b}	8.24^{ab}	9.97^{a}	9.43^{ab}	9.07^{ab}	1.17
ME intake MJ/kg$^{0.75}$/d	0.56^{a}	0.61^{a}	0.74^{a}	0.70^{a}	0.67^{a}	0.09
Intake N g/sheep/d	17.68^{b}	21.42^{b}	30.58^{a}	31.56^{a}	33.30^{a}	4.37
Urinary N excretion g/sheep/d	4.13^{b}	6.34^{ab}	8.0^{ab}	9.16^{ab}	10.18^{a}	2.56

Table 6. Weight gain and voluntary intake[37](VI) (kg DM animal/d/kg$^{0.75}$)

Treatments	Daily weight gain g/sheep	VI/kg/animal/day	VI/g kg$^{0.75}$
35,000 leucaena plants/ha	106	0.968	83.8
55,000 leucaena plants/ha	81	0.843	71.7

PODS OF TROPICAL TREES AS SOURCES OF FEED

In some regions among the tropical grasslands there are a variety of trees and shrubs which have excellent characteristics to be used for the feeding of ruminants as additives either as fibrous foliage, energy or protein sources, mainly during the dry season, when the grasses are scarce and of low nutritive value [40].

Chemical composition of the pods (Table 7) and the preference of ruminants for these resources may be considered as a partial substitute for the expensive starchy grains [41], which may lead to the design of feeding strategies which allow us to use these resources as additives to improve animal performance, thus optimizing the content of carbohydrates, proteins and minerals contained in tropical trees [42].

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PODS OF *E. CYCLOCARPUM* AS RUMINANT FEEDSTUFF

The legume tree *E. cyclocarpum*is widely available in several regions of Mexico and Central America. Similar to many other species of trees and shrubs, it has positive effects on the environment and it does supply foliages and pods which are readily consumed by different types of livestock. The yield of pods of *E. cyclocarpum* is 86 kg (fresh basis) per season [6], and these can be incorporated in "campesino" type, subsistence production systems, since that type of production systems can be considered as low-input demanding [16]. Chemical composition of fractions of the pods of *E. cyclocarpum* are given in Table 8, indicating that crude protein, ether extract and neutral detergent fiber are found in higher amounts in the seeds rather than in other components while the husks contain greater amounts of the fibrous components and lower amounts of protein. Rumen DM degradation of integral pods of *E. cyclocarpum* is similar that of the husks, however, husks have a lower potential degradation compared to that of the seeds (Table 9). The lower potential degradation of the integral pods and the husks may be influenced by the high content of ADF and lignin compared to those in the seeds which seem to contain high amounts of easily degradable monosaccharides (38.9%) and lower proportions of fibrous fractions [43], although under practical conditions seeds ingested by cattle, tend to pass intact throughout the GIT, being easily observed in the feces of the animals. Thus to optimize the utilization of the seeds it is necessary to ground them to a particle size of 3 mm to increase the surface area for the attachment of rumen bacteria.

Table 7. Chemical composition of pods

Chemical composition of pods of tropical trees (%)						
Species	CP	NDF	ADF	EE	TP	CT
A. pennatula	11.04	43.42	30.99	0.90	3.08	7.99
E. cyclocarpum	16.71	33.79	24.18	1.21	0.39	1.19
G. ulmifolia	6.53	46.98	32.11	2.02	0.32	0.71
M. pruriens	15.20	41.49	25.64	2.36	2.27	0.64
S. saman	13.94	23.78	18.47	1.28	0.50	1.09

CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; EE: ether extract; TP: total phenols; CT: condensed tannins.

Table 8. Chemical composition (g/kg DM) of integral pods of *E. cyclocarpum* [16]

Chemical composition	Fraction of the pods of *E. cyclocarpum*		
	Husk	Seeds	Integral pods
Crude protein	121. 9	243.5	167.2
Organic matter	960.1	961.6	957.3
Crude fiber	196.9	145.7	161.8
Ether extract	15.0	22.1	15.9
Neutral detergent fiber	325.1	399.3	353.6
Acid detergent fiber	255.6	214.8	231.7
Lignin	109.0	49.4	88.3
ME (MJ/kg DM)[7]			13.6
IVDOM (%)[7]			81.7

IVDOM: *in vitro* digestibility of organic matter.

Table 9. *In situ* DM degradation of integral pods of *E. cyclocarpum* [16]

Constant (g/kg DM)	Fraction of the pods of *E. cyclocarpum*			
	Husk	Seeds	Integral pods	SEM
T0	628.0[a]	443.0[c]	529.0[b]	7.04
a	701.3[a]	510.4[b]	567.3[b]	19.21
b	128.2[c]	424.2[a]	299.1[b]	13.04
a+b	829.5[b]	937.3[a]	866.4[b]	9.88
c	0.038[a]	0.028[a]	0.039[a]	0.06

[a,b]Means in the same row with different superscripts indicate statistical differences Tukey test (P<0.05).
[*]Adjusted to the exponential equation: $P=a+b\ (1-\exp^{-c*t})$[46]where a: Intercept of the exponential equation; b: Potentially degradable fraction; a+b: Potential degradation, c: Rate of degradation of fraction b (/h); T0: Washing loss.

Potential degradation of the pods of *E. cyclocarpum* (866.4 g/kg DM) is high, but the husks in this study have a higher potential degradation with respect to values reported previously [44] (829.5 *vs*. 639.0 g/kg DM). Even when complete pods of *E. cyclocarpum* contain a high concentration of ADF and lignin, the quickly degradable fraction and potential degradation are high, since it has been indicated [45] that as the level of the fibrous fractions is increased, rumen degradation is reduced, nonetheless, potential degradation of complete pods of *E. cyclocarpum* is superior to that determined for other tropical pods with good potential for ruminant feeding such as those of *P. saman* and *C. coronaria* (707 and 683 g/kg DM respectively).

In a digestion trial [47], levels of incorporation of ground pods of *E. cyclocarpum* (0, 20, 30, 40 and 50%) of ration DM by Pelibuey sheep were evaluated (Table 10). DM intakes registered were lower than those reported previously [7], who registered with levels of incorporation of 0, 12, 24 and 36% of ration DM, daily intakes of 105.7, 105.6, 110.0 and 114.8 $g/kg^{0.75}$ respectively. DM intakes of 73, 87, 88, 97 and 91 $g/kg^{0.75}$ for levels of incorporation of ground pods of 0, 20, 30 40 and 50% respectively, show a linear trend, indicating that as the level of *E. cyclocarpum* in the ration is raised, dry matter intake is consequently increased contrary to what was established by other authors [48], who evaluated

the intake of sheep fed different rations containing pods of *E. cyclocarpum* (0, 10, 20 and 30%) mixed with corn grain, corn straw, soybean meal and minerals with crude protein contents of 11.60, 13.78. 13.65 and 14.21%, registering DM intakes of 88.1, 79.3, 68.3 and 64.9 g/kg$^{0.75}$ respectively. The linear increase in intake observed in this trial [47] suggest that the quickly degradable fraction (567.3 g/kg DM) in complete pods of *E. cyclocarpum*, is immediately fermented avoiding physical fill of the rumen, decreasing rumen retention time and allowing the animal to consume greater amounts of the ration [9,50].

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Apparent digestibility of DM was reduced by 12.5% in the ration with 50% addition of ground *E. cyclocarpum* relative to the control treatment without the pods; this reduction may have been influenced by the increase in the concentration of ADF (38.2, 81.1, 99.8, 126.1 and 140.6), as the level of *E. cyclocarpum* was raised from 0 to 50%, due to the fact that the fibrous fraction of feedstuffs is the main factor constraining digestibility, existing a negative correlation among ADF concentration in feedstuffs and apparent digestibility [50, 51].

In ruminants, generally speaking, when feed intake is increased there occurs an increase in passage rate from the rumen, thus rumen microorganisms have less time to ferment structural carbohydrates in the rumen.

Therefore, it is likely that the linear increase in voluntary intake, was associated with the decrease in apparent digestibility as a result of the lower rumen retention time which impacts over a lower degradation of starchy and fibrous fractions of the ration [52,53,54].

OM digestibility in the experiment reported herein with a 40% addition of ground pods of *E. cyclocarpum* is similar to the *in vitro* digestibility reported [7] with a 36% addition (DM basis) of pods of *E. cyclocarpum* (723 *vs.* 733 g/kg DM, respectively) to a sheep ration.

OM digestibility shows the same trend towards a reduction as observed with DM digestibility, although this was only observed between the 0 and the 50% addition (highest level) of pods of *E. cyclocarpum* in the ration of sheep. This reduction in OM digestibility may be related to the defaunating effect of steroidal saponins contained in *E. cyclocarpum*. This agrees with the 22% reduction in OM digestibility in rations which contained 187 g/kgDM as foliage of *E. cyclocarpum* with respect to a control ration without *E. cyclocarpum* fed to sheep [55].

Table 10. Voluntary intake and apparent digestibility of rations containing increasing levels of ground pods of *E. cyclocarpum* by hair sheep [16]

Item	Level of incorporation of pods of *E. cyclocarpum*				
	0	20	30	40	50
Intake (g/kg$^{0.75}$)					
DM	73	87	88	94	91.0
OM	69	81	83	88	85.0
ME	0.9	1	1	1	0.9
Apparent digestibility (g/kg$^{0.75}$ DM)					
DM	780	772	758	722	710
OM	795	776	760	723	709
Intake (g/kg$^{0.75}$)					
DDM	58.9	67.3	66.9	67.6	64.7
DOM	55.8	63.5	62.7	63.2	60.2

DDM: digestible dry matter;DOM: digestible organic matter.

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PRODUCTIVE PERFORMANCE OF SHEEP FED PODS OF TROPICAL LEGUMES

One of the best expressions of the nutritive value of any feedstuff for ruminants is the productive performance obtained when it is fed alone or mixed with other components of an integral ration. That is daily weight gain, milk production, wool production. In a trial carried out at this laboratory [41] with Pelibuey sheep housed in individual cages during 71 days (15 days adaptation followed by 56 days for measurements) the effect of adding 45% of pods of *Acacia pennatula* or *Enterolobium cyclocarpum* in a complete ration on DM intake, daily weight gain and feed conversion efficiency was evaluated. The control ration consisted of an isoenergetic and isoproteic mixture based on sorghum grain, soybean meal, hay of *Brachiaria brizantha*, soybean husks, vegetal oil, cane molasses and a mineral mixture. Experimental rations consisted of a substitution of 45% of the grains by ground pods of *A. pennatula* and *E. cyclocarpum*. Rations were formulated according to nutrient requirements [56] for entire male sheep of 20 kg live weight, to cover maintenance and weight gains estimated to be 250 g/animal/d. Rations had a metabolizable protein (MP) content of 110 g/kgDM and a metabolizable energy (ME) concentration of 11.4 MJ/kg DM. Results are shown in Table 11. There were no significant differences ($P>0.05$) in the initial and final weights of the sheep, indicating that weight changes were similar among the three groups of animals. DM intake of sheep showed statistically significant differences ($P<0.05$) between the *A. pennatula* group with respect to the control, but it was similar to the ration with *E. cyclocarpum*. When intake was expressed as g DM/kg$^{0.75}$, there were also significant differences ($P<0.05$), the greater intake being for *A. pennatula*, although there were no differences ($P > 0.05$) between the control treatment and that with *E. cyclocarpum*. DM intakes (g DM/kg$^{0.75}$) recorded in this study are rather high and similar to those reported by others [7] of 105.7 g for the control and of 114.8 g for the level of incorporation of 36% (DM basis) of complete pods of *E. cyclocarpum* in Katahdin x Blackbelly sheep. Pelibuey sheep fed increasing levels (0, 20, 30, 40 and 50%) of ground pods *E. cyclocarpum* in the ration, gave DM intakes of 73, 87, 88, 94 and 91 g/kg$^{0.75}$ respectively, indicating that as the level of inclusion of the ground pods increases, the amount of feed consumed is also raised [47]. Weight gain and feed conversion efficiency showed no significant differences ($P>0.05$) among treatments, strongly suggesting that pods of tropical legumes have the potential to substitute partial feedstuffs with a high content of starch.

Other authors [3] who evaluated four commercial rations based on grains with sheep, found that weight gains fluctuated between 202 and 285 g/d with a feed conversion efficiency among 5.4 and 3.9 per kg of feed respectively, this suggests that partial replacement of concentrates with tropical pods from legume trees can maintain a reasonable daily weight gain and feed conversion efficiency. Performance data has been reported [7] similar to that in this report for tropical sheep fed rations with a smaller incorporation of ground pods of *E. cyclocarpum*, while there are no comparative data for performance of sheep fed high levels of ground *A. pennatula*. Nonetheless, preliminary data from this laboratory [41] reported that sheep preference for ground pods of *A. pennatula* is rather high. Recent work at this laboratory [57] evaluated the effect of the addition of 50% of ration DM as ground pods of *E. cyclocarpum* against a balanced pelleted ration (17% CP) fed to Pelibuey sheep in a commercial farm in South Mexico.

Table 11. Productive performance of hair sheep fed pods of two tropical legumes (Briceño et al., unpublished data)

	Control (n=6)	*A. pennatula* (n=6)	*E. cyclocarpum* (n=6)
Initial weight (kg)	21.0[a]	19.5[a]	20.1[a]
Final weight (kg)	35.5[a]	32.0[a]	32.5[a]
DM intake (g/d)	1131.5[b]	1334.8 [a]	1180.8[ab]
DM intake g/kg$^{0.75}$/d)	102.1[b]	128.3[a]	111.6[b]
Weight gain (g/d)	267.0[a]	240.5[a]	233.8[a]
Feed conversion efficiency	4.5[b]	5.7[a]	5.2[ab]

Table 12. Productive performance of Pelibuey sheep fed pods of *E. cyclocarpum* in a commercial farm in Southern Mexico[57]

Treatments	Daily weight gain (g)	DMI (kg/day)	Feed conversion efficiency
Ration with 50% ground pods of *E. cyclocarpum*	239	0.923	4.6
Commercial pelleted ration	251	0.776	4.3

Results presented in Table 12, are similar to those reported by other workers [41, 3]. Performance data indicate that the addition of ground pods of tropical legumes to rations of tropical sheep does not affect productive performance obtaining reasonable weight gains, reducing the incorporation of expensive grains and therefore improving profitability for the small producer.

CONCLUSION

Foliage and pods of tropical trees and shrubs represent a good source of food for ruminants that can be employed more intensively to improve profitability of small farmers in developing countries. Foliage and pods of tropical trees and shrubs provide fermentable energy (ATP) and nitrogen (NH_3) to the rumen microbes to fuel microbial growth in the reticulo-rumen, which in turn helps to ferment dietary organic matter which provides volatile fatty acids to be used by the host for maintenance and production. Several of these pods and foliage contain secondary metabolites [58] which may have positive effects on the growth of the ruminant by providing more dietary protein at the post-ruminal tract or by reducing methane production in the rumen (i.e. condensed tannins). Other metabolites (*i.e.,* saponins) may reduce rumen protozoa counts [55] and then methane production [59]. Condensed tannins may also impair rumen biohydrogenation of unsaturated fatty acids [60] making available a larger amount of beneficial acids such as vaccenic and rumenic acids which could modify the pattern of fatty acids in ruminant meat and milk [61] with potentially positive effects for human health. There are various possibilities towards increasing the concentration of unsaturated fatty acids in ruminant products thus lending added value to various ruminant products, improving profitability for the ruminant producer which is highly compromised at present by the increase in the costs of concentrate feedstuffs.

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Chapter 5

UTILIZATION OF EXOGENOUS FIBROLYTIC ENZYMES IN RUMINANT FEEDING

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ABSTRACT

Added fibrolytic enzymes (*e.g*. cellulases, xylanases) to diets for ruminants have been viewed with considerable skepticism for years.

Despite the considerable number of studies conducted recently, the mechanisms by which fibrolytic enzymes could improve fiber digestion in the rumen have not been clearly elucidated and seem to be affected by several factors such as the type of enzyme and the nature of the substrate.

Inconsistent responses are thought to be due in part to differences in physical and chemical conditions under which the enzymes have been evaluated; for example, several studies showed that the highest activity of carbohydrate degrading enzymes was at pH from 3 to 7 for β-glucanase and from 6 to 7 for xylanase.

It suggests that these enzymes seem to work better at close to neutral ruminal pH conditions.

In this chapter, we review some of the most relevant aspects about exogenous fibrolytic enzymes and its important influence over ruminant nutrition.

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ENZYMES OVERVIEW

With the discovery of the enzyme digestion in stomach (from 1760 to 1825) the first experiments on chemical catalysis were carried out.

In 1860, Pausteur postulated that fermentation was produced by the action of enzymes and that they were related to the structure and life of the yeast cells. These findings demonstrated that enzymes catalyze an energy-producing metabolic pathway, and can also act independently of the cellular structure; this was the first step toward descriptive biology through the understanding of biological processes. Pasteur's findings in enzymatic reactions facilitated progress within the small-scale manufacture of the bio-industry at that time [43, 73]. Summer, in 1926, isolated for the first time an enzyme in crystalline form. This enzyme was urease, isolated from *Cannavalia enzyformis* extracts and its crystals were formed by proteins. Noorthrop (from 1930 to 1936) isolated pepsin, trypsin and chymotrypsin; however, the proteic nature of these enzymes could not be established.

Starting in 1980, findings on enzyme reactions have resulted in new lines of research in this field. Among these findings are those related to the genetic control of enzyme synthesis, the self-regulating nature of many enzyme systems and the role of enzymes in development and differentiation [43]. The first attempt to use enzymes for industrial purposes was at the end of the past century. However, results were disappointing, mostly due to the lack of knowledge of enzymatic activity. During last 30 years, greater understanding of enzyme kinetics has allowed a greater use of enzymes in several industries due to the following [73]:

1) Come from natural sources and are non-toxic;
2) Have specific activity;
3) Work better in specific conditions (moderate temperature, wide pH range), no special equipment is needed;
4) Fast activity at relatively low concentrations; their reaction speed can be controlled by adjusting temperature, pH, and concentration;
5) Are easily inactivated when the reaction has reached the desired point.

Enzymes can be incorporated in feedstuffs in order to solve specific nutritional problems (Table 1). Several trials have demonstrated that enzymes application in feed improves animal performance and reduces environmental impact of animal production.

Enzyme activity is usually measured in specific conditions (controlled) on a specified period of time, and it can be evaluated in two ways:

a) Determining the substrate disappearance;
b) Determining the product production over a specific period of time.

The results are commonly expressed in units as gram per enzyme. The activity level of the enzyme is mainly influenced by temperature and pH as well as the specific substrate features and by the moisture in the used feed. From 200 known enzymes, only 20 are used in commercial form [53, 74].

Table 1. Objectives of enzyme supplementation in animal diets

Remove or destroy anti-nutritional factors.
Improve digestibility.
Turn certain nutrients biologically available.
Reduce the pollution impact from animal excreta.

Adapted from Sears and Walsh [73].

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USE OF EXOGENOUS ENZYMES IN RUMINANTS

The rumen is a complex microbial ecosystem and has two basic functions:

a) Release energy from carbohydrate sources, which can be used by the animal;
b) Turn non-protein nitrogen into microbial protein [3].

Ruminant animals provide bacteria with an adequate environment to live and in return bacteria provide to the animal with end products of fermentation (volatile fatty acids (VFA) and amino acids). The rumen has different types of bacteria which are responsible for producing the called "microbial enzymes". The predominant cellulolytic bacteria in rumen are *Ruminococcus albus*, *Ruminococcus flavefaciens,* and *Fibrobacter succinogenes. Butyrivibrio fibrisolvens* is also a cellulolytic bacteria, but its ability to degrade cellulose is limited. Some protozoa and fungi are considered capable to degrade cellulose to a lesser than cellulolytic bacteria [21, 71]. The main amilolytic microbes in rumen are *Ruminobacter amylophilus, Provetella ruminicola, Streptococcus bovis, Succinomonas amylolitica* and rumen protozoa. The ruminal fungus *Neocallimastrix frontalis* has possesses an α-amylase enzyme, but its importance in starch digestion is unknown [52].

Forages are generally the largest and most economical energy source for ruminants. Fiber from forages is composed primarily by cellulose and hemicellulose, which are digested by the cellulase and xylanase produced by the ruminal bacteria and protozoa [72]. It has been observed that diets based on forages and rapidly fermentable carbohydrates; adversely affect fiber digestion in rumen, due to a decrease in fibrolytic activity of microorganisms due to changes in microbial population and rumen environment [47, 81]. One of the most important alterations in ruminal environment that may affect fiber digestibility is pH. Dry matter digestibility of forages considerably decreases with increased dietary starches due to reduction in ruminal pH [71]. Reduced rumen pH decreases the activity and/or the number of cellulolytic micro-organisms. *Fibrobacter succinogenes* does not tolerate a pH lower than 5.9, *Butyrivibrio fibrosolvens* is more tolerant to changes in pH; however, most species have problems tolerating a ruminal pH below 5.7 [71]. Fiber degradation rate, also, depends on the forage offered to the animal, and even of the nature of their cell wall polysaccharides [58]. The cell wall is composed of cellulose, hemicellulose, pectin, structural proteins and lignin. The principal phenolic compound in forage is lignin, which is a three-dimensional polymer formed from fenilpropane structures. This polymer provides stiffness to the plants, joins vegetal cells, decreases the water permeability through the cell walls, and protects the plant against microbial attack [36, 55, 61].

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Since the initial assessment that the digestive process in all animals involves the breakdown of macro-nutrients by endogenous enzymes, nutritionists have tried to modify this process through the use of exogenous enzymes. The use of exogenous enzymes results in increased productivity and reduced costs in animal feeding, by reducing the use of grain due to the greater extraction of energy from the fibrous materials [3, 53]. Although there is an ample knowledge of the rumen and enzyme functions, attempts to modify its conditions have been less spectacular than those observed in other species, as for example in broiler chickens. This is due to the complexity of the rumen and by the high cost for the use of sheep or cattle as experimental units in an investigation. Nevertheless, the use of exogenous enzymes as an additive in ruminant diets is justified by:

1) The digestibility of the dry matter in ruminants is considerably lower than 100%;
2) Because of the use of low-quality feeds in ruminant diets, the exogenous enzymes can promote degradation of macronutrients in the rumen or anywhere in the digestive tract [3, 82].

The application of exogenous enzymes in ruminant nutrition can be observed in two ways:

1) As agents that improve the process of silage;
2) For its ability to act immediately before feed intake or at some point during its passage through the digestive tract of the animal.

Enzymes are generally used to improve the nutritional value of feed for nonruminant or as additives to silages; but they are not used routinely in diets for ruminants, because as proteins they would be rapidly degraded by rumen microorganisms. Microbial enzymes have been used by more than 40 years in animal feeding and are produced from specific cultures of bacteria or fungi. The selection of natural mutations and more recently by intra and inter-generic gene manipulation can make the microbes to produce large quantities of a specific enzyme or a group of enzymes. However, there are enzymes that have inappropriate activities, especially those from impure products [36]. The fibrolytic activity within the environment of the rumen is usually very high, and presumably difficult to increase by the simple addition of exogenous enzymes [14]. Some changes in rumen activity have been associated with the application of exogenous fibrolytic enzymes (Table 2).

Studies with enzyme preparations with hemicellulolytic, cellulolytic, amilolytic, and proteolytic activity that might improve considerably feed digestibility and animal response production performance were conducted in the 1960s. In ruminants, the results were inconsistent, and together with the high cost of enzymes and the potential to improve the animal response with other technologies, the use of enzymes in ruminant was abandoned. However, given the significantly increased cost of animal production, and the addition of a new generation and more available enzymatic preparations, the interest in using exogenous enzymes in ruminants increased despite inconsistent results [48, 81, 82].

The inconsistency in the results and different responses to exogenous enzymes has been attributed to several factors: the composition of the diet, the type of preparation, activity, and quantity and stability of the enzyme. It has been found that the response to enzyme additions varies with the origin and protection of the enzyme.

Table 2. Observed changes in ruminal activity associated with the addition of exogenous enzymes

Increased digestion of dry matter.
Increased neutral detergent fiber digestion.
Increased production of volatile fatty acids.
Increased carbohydrate use.
Reduced acetate:propionate ratio.
Altered ruminal pH.
Improved microbial protein synthesis.
Increased ammonia nitrogen production.
Increased urease activity.

Adapted from Dawson and Tricarico [25].

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The method of implementation is also an important factor; for example, applying the enzyme to forage before consumption improves digestibility due to different mechanisms. These mechanisms may include direct hydrolysis, improvement in the microbial attack, changes in the intestine viscosity, complementary action with ruminal enzymes, changes on the site of the nutrient digestion, and even higher feed palatability and changes in feed intake [22-24, 40, 51, 57, 66, 83]. It has also been observed that the direct addition of fibrolytic enzymes into feed of dairy cows increases fat, protein, and milk production, although these results have only been observed with a certain amount of enzyme [45, 48, 81, 82]. Other factors affecting the effectiveness of the exogenous enzymes are as follows: the level of humidity of the substrate, because differences have been found when apply to forage in dry, wet, or ensiled; the time required for the interaction of the enzyme with the substrate or the time between treatment and consumption; the physico-chemical characteristics of the cell wall of the substrate, the temperature at the time of treatment, energy balance and the ability of the animal to respond to increased available energy [18, 48, 81, 82].

MODE OF ACTION OF EXOGENOUS FIBROLYTIC ENZYMES IN RUMINANTS

Enzymes are proteins found in nature and that catalyze chemical reactions in biological systems. They are involved in the digestion of all complex molecules in feed to their most basic chemical constituents (*e.g.* glucose, amino acids), without them feed would remain undigested in the animal [41]. The knowledge of the exact mechanism of action of fibrolytic enzymes is complicated by three main factors:

1) Feedstuffs are structurally very complex;
2) Enzyme products are mixtures of enzymes which have many different activities, each of which different optimal conditions and specifications; and
3) The ruminal fluid is an ecosystem with hundreds of microbial species and their enzymes.

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Beauchemin *et al.* [15] mentioned that the addition of enzymes to improved total digestibility in the digestive tract of the starch (5%), NDF (8%) and ADF (11%) indicating that its effects are mainly at the intestinal level.

It is possible that the enzymes will attach to substrates prior the introduction to the rumen and prevent degradation by proteases [40] to pass into the small intestine, where can exert their action on the available substrates [57]. Enzymes produced by the fungus *Trichoderma* are relatively stable in rumen and resist the microbial degradation for a time sufficiently long to act in the rumen. Exogenous enzymes are more affected by gastrointestinal proteases and the pH of the abomasum, than by ruminal proteases. In contrast, Yang *et al*. [82] did not observe effects of enzymes either in the viscosity of the content or on the digestibility of nutrients in duodenum, concluding that the improvement in digestion is not due to the post-ruminal effect. Improvements in milk production have been attributed to the effect of enzymes on the digestibility of nutrients in the total digestive tract. Different mechanisms have been proposed to explain improved digestibility:

1) Direct hydrolysis of feed;
2) Improvement in the microbial fixation;
3) Changes in the viscosity of the intestine;
4) Complementary action with the microbial enzymes; and
5) Improvement of the digestion [15].

The proposed mechanisms of action by which exogenous fibrolytic enzymes improve the utilization of nutrients in the digestive tract of the ruminant animal are shown in Figure 1.

FIBROLYTIC ENZYMES

Digestive enzymes are essential for animals, because the complex foods are not absorbed in the digestive tract, but degraded to simpler molecules. The digestion process is complex and often involves multi-components enzymes; for example, a single preparation of cellulase enzyme complex contains endo and exo β-1, 4 glucanase, β glucosidase and cellobiase. Hemicellulase preparations are even more complex. Enzymes, also, have optimal pH and temperature at which they are more effective. The cellulase enzyme complex of *Trichoderma* fungi species has an optimum temperature and pH of 50 ^{o}C and 4.5, respectively.

Some digestive enzymes have been used in diets for poultry and pigs improving their productive performance. However, but these have not been traditionally used in diets fed to ruminants [3], mainly due to the fact that they are proteins and they could be degraded by proteases ruminal or inactivated by proteases in the small intestine [41]. Nevertheless, recently there is a growing interest in the use of enzymes in ruminant diets, most of the research on enzymes in ruminant diets has focused mainly on the use of the fibrolytic enzymes such as cellulases (degrade cellulose) and hemicelluloses (or xylanases that degrade hemicellulose). Exogenous enzymes for ruminants are concentrated extracts obtained from fermentation with fungi mainly *Trichoderma longibrachium*, *Aspergillus Niger*, or *A. orizae* and bacteria *Bacillus spp*. The extracts do not contain microbial cells, because they are removed from the fermentation, and finally concentrated and purified [10]. Fungi are

cosmopolitan; notable for its rapid growth and high capacity for using various substrates. The aerobic fungus *Trichoderma* produces large amounts of extracellular xylanases in both, steady and solid cultures.

Commercial products of enzymes contain a single settlement of enzymatic activities. For example, a preparation of enzyme with predominant xylanase activity could also have significant protease, cellulase, pectinase, β-glucanase, or other enzymatic activities. The activity of enzymes can be tested using an *in vitro* method, measuring the production of final products such as reducing sugars or amino acids per unit of time, using a specific substrate (usually purified), under defined conditions.

The typical methods used for feeds assessing, as the *in vitro* and *in situ* tests, can be used to evaluate the enzymes in relation to different substrates, but the experience has shown that these results often do not agree with the results of feeding trials [70]. On the other hand, the percentage for the inclusion of exogenous enzymes in diet for ruminants usually ranges from 0.01 to 1% of the diet; contributing with about 10 to 100 times greater fibrolytic activity per gram of feed, when used as an additive for silage.

Based on the estimated average of the fibrolytic activity that normally is present in the fluid fraction of the rumen, it is estimated that supplemented enzymes can contribute up to 15% of the total fibrolytic activity. However, commercial enzyme activity is measured at optimum pH, which differs from that found in the ruminal fluid. In addition, the contribution of exogenous enzymes on fibrolytic activity is difficult to estimate, but is also low in relation to the rumen [69]. A good product of enzyme in feed should contain a mixture of enzymes such as effective in a range of feeding conditions. Maybe an enzyme preparation can be effective in a feed and does not cause effect in another. However, enzyme preparations have been shown to be effective in a wide range of diets containing alfalfa hay, silages and grains [from oats and corn; 70].

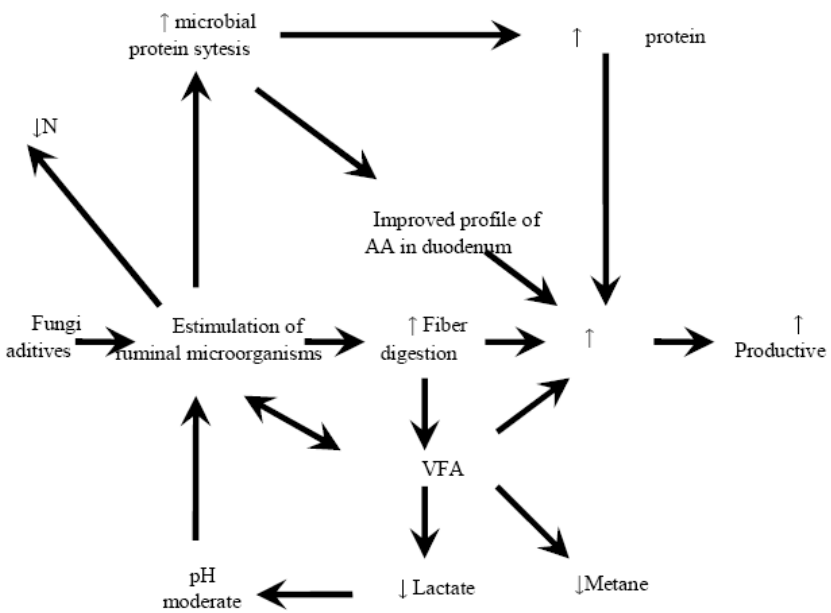


Figure 1. Mechanisms of action proposed for exogenous fibrolytic enzymes in diets for ruminants [Adapted from Kung, 41].

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Concentration of Enzyme

The optimum enzyme concentration inclusion depends on the substrate availability, which determines the optimal application percentage of enzyme preparations for a particular feed substrate [14]. For example, the average daily gain (ADG) increased with low enzyme concentration addition when alfalfa hay was fed to growing cattle, but not with high concentration inclusion. Compared with control diet the ADG was increased by 30% with the addition of 4X the amount of enzyme. For fleo hay (*Phleum pratense*), high-dose enzyme inclusion (16 X) improved ADG by 36%, mainly due to an increase in fiber digestibility of 17%. This improvement in ADG did not change feed intake; however, feed conversion was reduced by 20%. It was not observed response in ADG with low or moderate doses on fleo hay diets, which contrast to what is observed in alfalfa hay based diets. Treacher *et al*. [78] reported a low or negative animal response to high concentration of enzymes, besides not being economically viable.

USE OF EXOGENOUS FIBROLYTIC ENZYMES ON THE FEEDING OF RUMINANTS

A commercial enzyme containing endo-cellulases, exo-glucanases, xylanases, and β-glucanase, was added to dairy cows diets (1.22 L t^{-1} enzyme and 3.67 L enzyme t^{-1}) in high and low doses [12]. Both doses increased dry matter intake, which may be due to a possible increase in palatability or a higher rate of passage of feed through the digestive tract. The time used in feed intake per unit of fiber was reduced, which suggests that the enzyme causes a previous digestion of feed, facilitating chewing. The DM, OM, and NDF digestibility was improved with the low dose of enzyme; possibly, a high concentration of enzyme can compete with ruminal bacteria for the binding sites in particles of feed, causing a reduction in total endogenous bacterial activity. Ammonia concentration decreased with the low-dose enzyme, due to the increase in rumen of available carbohydrates of slow digestion caused by the enzymes addition.

These results demonstrate that a low enzyme concentration can improve the intake and digestibility of the dry matter; while, a high level of enzyme only improves the feed intake. Bowman *et al*. [17] added a fibrolytic enzyme (Promote®) to a diet for dairy cows (55% forage), and did not record changes in rumination time, feed palatability, saliva production or ruminal pH. Yang *et al.* [82] added 1 g/kg DM to the diet and 0, 1 and 2 g of fibrolytic enzyme (Pro-Mote®)/kg DM hay on a diet (45% supplement, 10% barley silage, and 45% of alfalfa hay) for dairy cows. There were no differences in feed intake and ruminal digestibility or duodenal flow of OM, NDF, ADF, and N. However, they reported increased ruminal fermentation and synthesis of microbial protein with enzyme at 2 g/kg hay and 1 g/kg total dry matter diet. With the highest doses of enzymes, the milk production increased due to changes in digestibility. In contrast, exogenous fibrolytic enzyme added to high-concentrated diets to sheep, did not change intake and digestibility of dry matter in total digestive tract [81].

In growing steers fed alfalfa with 4733 international units (IU) of xylanases, increased weight gain, ADF digestibility and improved feed efficiency; fleo hay (*Phleum pratense*)

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treated with 12000 UI of xylanases improve weight gain, ADF digestibility and feed efficiency. When the same enzyme was fed with barley silage (*Hordeum vulgare*) decreased feed intake and ADF digestibility were recorded [14]. These results are consistent with those obtained by Feng *et al*. [28, 29] who added fibrolytic enzymes directly to hay before being consumed, and observed increased feed intake and rumen and total tract digestibility. The addition of a fibrolytic enzyme (Fibrozyme®) to corn silage or Guinea grass (*Panicum maximun*) for dairy cows did not affect crude protein and NDF intake, but there was a slight increase in dry matter intake in both forages [26]. In agreement, Pinos *et al*. [64] added 5 g Fibrozyme® directly to the rumen of native sheep and found an increase on *in vivo* digestibility of CP and hemicellulose in diets with alfalfa hay. When the enzyme was fed to diets with alfalfa and ballico hay, N intake, fecal N, retained N, and *in situ* disappearance of DM, NDF, ADF and hemicelluloses were increased. An exogenous enzyme complex added to the rumen of dairy cows increased the fibrolytic activity in rumen (especially that of xylanases), partially escaped reticulum-rumen showed increased activity in duodenum [38]. The use of an exogenous enzyme complex also stimulated the growth of cellulolytic bacterium without a pre-feeding feed-enzyme interaction [31]. Fibrolytic enzymes may produce a better response when added to feed, because they are more resistant to ruminal proteolysis and they have greater potential to be active in the small intestine.

Spraying corn silage and lucerne hay with a liquid enzyme mixture (with cellulases and xylanases) to a 50:50 (forage: concentrated) diet for dairy cows, there was no effect on forage fiber, but it improved milk production with a dose of 3500 and 16 000 UI/kg DM of forage (of carboxymethyl cellulase and xylanases). Cellulases, probably due to their low molecular weight, can be located in small pores of forage and are inaccessible to other enzymes of higher molecular weight, decreasing the synergy with other cellulases, (endoglucanase and celobiohidrolase), and the final result is a decrease in the hydrolysis of cellulose. Also, high doses of fibrolytic enzymes may decrease forage chewing, decreasing saliva production, causing a reduction in ruminal pH and therefore fiber digestion, which reduces milk production. Thus, treatment with high doses of an enzyme may not have a beneficial effect; in addition, various combinations of enzymes may be more efficient than the addition of a single enzyme [40]. A study was conducted to evaluate the effect of the method of delivering a fibrolytic enzyme (cellulase and xylanases; Grasszyme®) on the digestive characteristics of calves (diet 70% forage and 30% barley; 44). The application or not of the enzyme to forage or barley at different times before being offered, and enzyme added in rumen 2 h after feeding was evaluated. Only the enzymes via ruminal caused a minor animal response, possibly because the enzymes are digested in the rumen or remain suspended in the liquid fraction of the rumen and leave the rumen without having enough time in contact with the fibrous fraction to hydrolyze it.

Lewis *et al.* [45] evaluated the effect of an enzyme solution (cellulase and xylanases) sprayed to the forage fraction of a total mixed ration for Holstein cows, resulting in increased milk production and fat and protein content. Schingoethe *et al.* [72] applied an enzyme mixture of cellulases and xylanases (0.7, 1 and 1.5 L enzyme t^{-1} DM forage) to forage (60% corn silage and 40% alfalfa hay) of an total mixed ration before being offered to dairy cows, and recorded increased total milk and corrected fat (3.5%) in cows that received diets treated with enzyme. These results may be due to a pre-degradation of fiber, that reduces NDF content in feed, which can increase the soluble fraction due to the direct addition of the

enzyme [4, 33, 34, 37], however, these results differ from those reported by Alvarez *et al*. [2] using 2 g/kg DM.

Peters *et al*. [62] applied an enzymatic product with xylanases and cellulases (9000 U endo-1, 4-β-glucanase, 24000 U endo-1, 3 (4) - β-glucanase and 40000 U 1, 4-β xylanase per ml) to a mixture of 50% concentrate, 30% of corn silage and 20% grass silage, at approximately 6.2 ml/kg TMR. They did not find changes on any parameter for the use of this enzyme complex. On the other hand, the addition (spraying) of two fibrolytic enzyme complex from *Trichoderma longibrachiatum* to diets with more than 60% of forage (corn silage and alfalfa hay) for dairy cows resulted in no change in milk production, or fat and protein in milk [79]. In a different study conducted by Sutton *et al*. [75], they used the same enzyme complex than Vicini *et al*. [79; from *Trichoderma longibrachiatum*] applied to a total mixed ration (57% of forage) or to concentrate or added directly to the rumen of four cows equipped with ruminal and duodenal cannulas. The three application methods had similar effects, but when the enzyme was added to the total mixed ration, milk production increased, possibly due to an increase in the consumption of digestible OM.

Few studies have focused on evaluating the effect of adding fibrolytic enzyme complex in beef cattle. Titi and Tabbaa [77] showed the benefits of both fibrolytic enzymes in digestion and weight gain of replacement heifers. It was observed that alfalfa hay fed with an intermediate dose of enzyme resulted in increased DMI (14%), ADG (30%) and feed conversion (16%). Timothy grass and high doses of enzymes feed to steers resulted in 5% improvement in DMI, ADG (33%) and feed conversion (23%). Reduced doses of enzyme also resulted in improved DMI (8%), ADG (9%) and conversion food (3%) to a lesser extent when added to silage barley. McAllister *et al.* [49] recorded improvements in DMI in steers fed silage supplemented with enzymes, but there was a reduction in DMI of 2.6 and 6.3% by adding enzymes to barley silage. Feng *et al*., [27] reported improvements in CMS from 2 to 12% by the addition of enzymes to forage dry grass immediately before feeding steers. Beauchemin *et al.* [13] observed improvements in feed conversion (12%) in steers fed diets containing barley treated with highly active xylanase but not with high activity cellulase. No effects were detected in animals fed corn diets supplemented with enzymes, except for feed conversion that improved from 4 to 7%. Beauchemin *et al.* [11] reported increased ADG (9%) and feed conversion (10%), but not DMI in heifers fed enzymes added to the concentrate supplement. Moreover, Zinn and Salinas [87] reported no change on ruminal pH, ruminal microbial efficiency or total ruminal digestion of organic matter or starch. Fibrozyme® supplementation increased ruminal digestion of NDF (23%) and N (5%). The total tract digestion of NDF and N was similar among treatments, but on growth performance of fattening calves for a period of 64 d supply, Fibrozyme® supplementation increased the final weight (3%), ADG (6%), and DMI (4.5%).

Zobell *et al.* [89] observed that in growing steers feeding a diet with 65% forage and supplemented with enzymes, showed numerical reduced feed conversion in the first 28 d. The carcass weight, rib area, back fat and growth performance, were also unaffected by the addition of enzyme. However, it may have beneficial effects during the arrival period of the animals. Wang *et al.* [80] studied the effects of the addition of monensin, surfactant and fibrolytic enzymes in beef cattle, noting that enzymes improved ADG by 5% only during the adjustment period, but not during the finishing period. Moreover, Queiroz *et al.* [68] found no change in the productive response of young bulls by the addition of fibrolytic enzymes and yeast to diets including 65% corn silage and sorghum. In starting steers fed sugar cane and

urea as a supplement in a tropical environment, they observed an improvement in the ADG and sugar consumption and digestibility of both forages due to the addition of fibrolytic enzyme (Fibrozyme®; 35).

Basurto [9] added a liquid enzyme product, originated from *Trichoderma longibranchiatum*, to the forage and supplement for bulls in confinement, and found that the enzyme improved weight gain, feed conversion, and the economic return of investment. In growing-finishing rations, the addition of enzymes can improve the performance of fattening cattle in feedlot, due mainly to the increase in energy consumed through a better fibrolytic capacity in rumen [88]. In steers fed diets with short and large particle length of forage Carreón *et al.* [20], use of an exogenous fibrolytic enzyme mixture increased the potentially disappearance fraction and total disappearance of DM, starch, NDF and ADF of diets with both short and long particle length forage. The addition of enzymes can improve the productive response of the animal, independently of its effect on the fiber digestion, possibly by ruminal changes or alterations in the final products of fermentation. The addition of a preparation of enzyme from *Trichoderma longibrachiatum* in diets for dairy cows increased the number of ruminal bacteria that use hemicellulose and the secondary by-products of digestion of cellulose, increasing fiber and whole ration digestion [32, 34, 59].

On *in vitro* incubations with alfalfa hay and maize silage some enzymes exert part of its effect by removing the structural barriers that retard the microbial colonization of the digestible fraction, and increasing degradation. However, in some cases exogenous enzymes are effective only after adding ruminal liquid, suggesting that they require interaction with ruminal enzymes to degrade the forage faster and that there are a potential synergy between exogenous and ruminal enzymes [22, 23]. The addition of some fibrolytic enzymes may be beneficial to alter the microbial activity in the rumen, as well as to change ruminal fermentation [25].

An fibrolytic enzyme product (Pro-Mote®) applied in the aqueous form to the concentrate fraction of a total mixed ration for dairy cows, did not affect DM intake, starch digestibility was lower, but increased the digestion of NDF and ADF, and milk production. The later may be due to an increase in digestibility of nutrients in the entire digestive tract [69]. Krause *et al.* [39] used a fibrolytic enzyme product (Pro-Mote®) in steers fed with four diets (95% concentrate and 5% forage, 100 g enzyme L^{-1} adding 15 L t^{-1}), combined with silage and hay of barley; decreased the concentration of NDF and ADF of the diet, independently from the forage source, indicating a partial hydrolysis of the fiber because of the enzyme. The feed intake of DM, NDF and ADF, ruminal pH, VFA, digestion of the DM, OM, and NDF did not change, but increased digestion of ADF in diet added with enzyme, silage 55% and 14% barley hay. This increase in fiber digestion may be due to a higher ruminal digestion of the grain, because the husk of the barley limits the attack by rumen microorganisms to the highly digestible endosperm.

Steers fed diets based on corn stover treated with 3 g enzyme product (Fibrozyme®)/kg DM showed improved feed conversion and *in situ* digestibility of DM, NDF, ADF and starch in the diet with enzyme addition, but it did not change the ADG and DM intake [19]. These results are similar to those reported by Meneses *et al*. [54] who found no changes on *in situ* digestibility of DM, NDF and starch with addition of Fibrozyme®, despite improved digestibility of ADF. Yescas *et al.* [84] fed sheep a diet with 65% of forage treated with 1 g Fibrozyme®/kg DM and found no difference on *in situ* digestibility of DM, NDF, ADF and ruminal pH.

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An exogenous fibrolytic enzyme added to Bermuda grass did not change *in situ* digestibility of DM and NDF or the composition of the cell wall, concentration of *p*-cumaric and ferulic acid or final products of fermentation of silage [46]. In bulls fed with tropical grasses and sugar cane plus 15 g Fibrozyme® /animal/ d, there was no effect on the DM digestibility, total feed intake and weight gain, possibly because tropical forages require greater amount and frequency of enzyme administration to undergo changes [18]. With the addition of fibrolytic enzymes (Pro-Mote®) to diets of whole barley and barley without husks for dairy cows, Beauchemin *et al.* [15] found no effect on DM intake and ruminal digestion; however, diets with barley without husk and enzyme increased digestibility of OM, N, NDF, ADF and starch in total digestive tract. Additionally, the conversion of microbial N from $N\text{-}NH_3$ and the content of protein and fat in milk also increased.

Pinos *et al.* [63] incubated alfalfa hay or ballico with or without Fibrozyme®, and observed no changes in the disappearance of DM and OM in the first 12 h of incubation; however, the disappearance of NDF and ADF increased in both forages. In agreement, Pinos *et al.* [65] reported that fibrolytic enzyme would cause a positive effect on *in vitro* degradation of cell wall components. They also reported that enzyme was not digested or dissolved during first 12 h of *in vitro* incubation. These results are similar to that reported by Avellaneda *et al*. [5-8] incubate Guinea grass and *Brachiara* grass added with an exogenous enzyme mix (Fibrozyme®). They did not find changes on the *in vitro* digestibility of DM, but the NDF and ADF digestibility was improved in both forages, which suggests a positive effect of the enzyme in the cell wall degradation.

Hristov *et al.* [38] incubated two enzymatic complexes that degrade polysaccharides and found a marked resistance to the *in vitro* ruminal fermentation. Consistently, Mandebvu *et al*. [46] did not observe changes on the *in vitro* digestibility of DM and NDF of Bermuda grass treated with fibrolytic enzyme of cellulases, α-amylase and hemicelluloses. In contrast, a fibrolytic enzyme improved the *in vitro* NDF degradation in alfalfa hay due to an alteration in the structure of hay by action of the xylanases and esterase of the enzymatic complex [60]. The DM digestibility of hays of alfalfa or grass treated with a cellulolytic enzyme was improved [67], but in diets with silage and barley grain plus fibrolytic enzyme (Pro-Mote®), they observed that the *in vitro* NDF digestibility was not changed. However, the DM digestibility increased in the first hours of incubation, although in the next few hours there was no effect on the digestibility of nutrients, and therefore the enzyme would only improve the rate and not the degree of digestion [1, 16, 30]. An enzyme fibrolytic (Pro-Mote®) added to diets (50% of forage) for dairy cows, did not change the synthesis of microbial protein, $N\text{-}NH_3$, or VFA concentration, but increased the molar proportions of acetate and decreased propionate [83]. Incubated corn stalks treated with a fibrolytic enzyme improved the *in situ* digestibility of DM and NDF and increased VFA concentration [76].

The use of exogenous fibrolytic enzymes with other additives (*e.g*. yeast), has increased milk production, DM intake, and composition of milk, because the combination of these additives, favors cell walls hydrolysis and facilitates access to the main chains of cellulose polysaccharides [85, 86]. Montañez *et al.* [56] evaluated the effect of exogenous fibrolytic enzymes on ruminal fermentation and diet component disappearance in steers fed high-concentrate diets with sodium bicarbonate. Dry matter intake, ruminal pH, ammonia N, ruminal disappearance of dry matter and neutral detergent fiber of diets were not affected by enzymes or enzymes plus bicarbonate. Steers, spent more time ruminating and resting and less time eating than steers on the control diet with no enzyme added. Ruminal pH,

ammonia-N, and cellulolytic bacteria concentrations were greater in steers fed enzymes with bicarbonate than in steers fed the enzyme with no bicarbonate or not supplemented with enzyme or bicarbonate.

Products from fermentation of *Aspergillus oryzae*, *Bacillus subtilis*, *Lactobacillius acidophilus* and a yeast culture (*Saccharomyces cerevisiae*) mixed with an enzyme (α-amylase and small amounts of β-glucanase, cellulase and hemicelullase) added to the diet of dairy cows (21.2 g/d/animal) increased milk production, and decreased the percentage of fat without changing milk protein concentration [50]. A yeast culture (*Saccharomyces cerevisiae*) and enzymes (α-amylase, protease, and lipase) in diets for dairy cows did not change milk production, but increased the production of fat corrected milk [42].

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CONCLUSION

The use of exogenous fibrolytic enzymes in ruminant feeding can potentially improve the efficiency of feed utilization and cell wall digestion in forages, especially in confined dairy and beef cattle. However, the responses are inconsistent, possibly due to factors such as diet composition, method of application of the enzyme as well as enzymatic preparation, activity, concentration, and stability among other factors. More studies are needed to evaluate variables that have shown the better results and thereby encourage the use of exogenous fibrolytic enzymatic compounds in animal production system.

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Chapter 6

USES OF *SACCHAROMYCES CEREVISIAE* AS FEED ADDITIVE IN HORSE FEEDING

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ABSTRACT

Saccharomyces cerevisiae (Sc) has been used in equine nutrition for several decades. Several studies have found that administration of yeast to a horse's diet results in increased digestibility. Yeast helps to prevent reducing hindgut microbial disturbances related to sudden diet changes. It has been observed that addition of live yeast to a horse's diet increases lactobacilli in cecum and improves enzyme activity in cecum and colon. Some of the benefits of adding active live yeast to a horse's diet, can be explained by the regulation of Polysaccharidase activities involved in plant cell wall digestion and activities of glycoside hydrolases (α-l-arabinosidase, β-d-cellobiosidase, β-d-glucosidase,β-d-xylosidase) induced by the addition of Sc to the diet. This additive is also able to absorb feed- contaminating mycotoxins and may help reduce the incidence of cancerigenic, teratogenic, mutagenic, hepatotoxic, immunosuppressive, and brain, kidney, reproductive and digestive systems damage related to mycotoxicosis in horses, associated to with food poisoning. For all of these reasons, Sc could be used as a beneficial feed additive in horse diet.

INTRODUCTION

It has been a long time since yeast was first used for human biotechnological purposes. Yeast was first discovered by Egyptian bakers to improve flavor and consistency of bread. Since then, yeast has come along with human culture in some sort of biotechnological symbiosis. Some of the additional applications that have been found for yeast are the

manufacturing of wine, beer, and other spirituous beverages. Modern uses of yeasts includeing their use as bioreactors for the production of proteins, as models for genetic and metabolic studies and as prophylactic or therapeutic agents either for humans or animals.

More than 1,500 yeasts species are known, and there should be yet many species that remain to be discovered. However, from the vast number of species already described, probably the best known and most thoroughly studied species is *Saccharomyces cerevisiae* (Sc), which is nowadays most widely used [1].

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The Sc has been used as a feed additive in animal's' diets because it has been demonstrated that this yeast has beneficial effects on the digestive tract and the immune system, improving weight yield and reducing the frequency or severity of the diverse infectious diseases [2-4].

This yeast is a natural source of digestive enzymes, fatty acids, vitamins of the B complex and from unknown growth factors and aminoacids that explain some of its nutritional benefits.

It is also rich in oligosaccharides, such as mannose and beta-glucans, helping in preventing bacterial infections and to modulatinge the immune response of animals that have the yeast as a regular part of their diets. Beyond these attributes, it has been observed that yeasts can also have beneficial effect in reducing toxic effects in animals of mycotoxins present in contaminated grains [5-7].

In this chapter, some of the mechanisms through which Sc is able to improve horse nutrition and husbandry when it is supplemented in the diet will be discussed.

EFFECTS OF *SACCHAROMYCES CEREVISIAE* IN FORAGE DIGESTION

The hHorse is an herbivore whose diets includes a large proportion of fiber (at least 50%);, however, its digestive tract does not have specialized enzymes to digest such a large amount of fiber intake. Rather, the digestion of forages relies on microorganisms. The efficiency in fiber digestion of the microbiota depends on the source of fiber, other diet components and on the type of microorganisms populating the digestive tract of the animal [8,9].

It is well documented that the recommended diet for an individual horse depends mainly on the load of work, and the physiologic stage of the animal. For instance, high -performance sports horses, rapidly growing foals, pregnant mares or horses recovering from illness have an increased need forof protein and energy. In many cases, it is not possible to meet nutritional requirements with diets based on forage, and therefore it is necessary to supplement animal's meals with carbohydrate- rich diets (mainly grains). Since the digestive tract of the horse does not evolve to digest such amounts of starch, many fermentative problems may arise making the horse more susceptible to recurrent colic and laminitis. These digestive problems may be prevented or controlled with the use of some prebiotics and/or probiotics helping to maintain a healthy hindgut ecosystem [10].

Several studies have found that administration of active live yeast (Sc) to horses results in increased digestibility. For instance Agazzi *et al.* [11] and Jouany *et al.* [8] found an improved digestion of dry matter, organic matter, neutral detergent fiber, and acid detergent

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fiber in mature horses, as well as an stimulated dry matter and neutral detergent fiber intakes. They propose the strategy of using Sc to stimulate cellulose digestion and improve the nutritional status of horses either under high- fiber or high- starch diets. Supplementation of meals with Sc appears to modify pH, concentrations of lactic acid and ammonia, molar percentages of acetate and butyrate with high- starch diet and (acetate -butyrate)/propionate ratio when high- fiber diet is fed. The effects of the Sc supplementation are greater in the cecum than in the colon, which coincides with the abundance of yeast cells. When the digestion of starch in the small intestine is saturated, the effect of the addition of Sc appears to limit the extent of undesirable changes in the intestinal ecosystem [10]. Furthermore, Glade and Biiessik [12] found that yeast supplement increases daily net nitrogen retention as well as cellulose and hemicelluloses digestibility, which is likely due to an increment of microbial production of aminoacids and volatile fat acids.

Yeast helps to prevent reducing hindgut microbial disturbances related to sudden diet changes. For example, the addition of barley to the diet causes substantial changes in the colonic microflora, such as increases in the concentration in total anaerobes, *lactobacilli*, *streptococci*, and lactate-utilizing bacteria. Yeast cell wall contains *fructooligosaccharides*, which may help reduce barley intake-related changes preventing increments in *Lactobacillus* and *Streptococcus*. In an experiment by Respondek *et al.* [13], it was found that although the colonic D-lactate concentration increased after a meal of barley, it did not accumulate with Sc *fructooligosccharides* (scFOS) supplement. These data indicates that a scFOS supplement is effective in reducing disruptions of the microbial populations in the equine hindgut under stressful situations like acute starch overloads.

The mechanisms of action of live yeast or yeast derivatives have not been fully understood. However, it has been observed that addition of live yeast to horse's diet increases lactobacilli in cecum and improves enzyme activity in cecum and colon. Some of the benefits of adding active live yeast to horse's diet, can be explained by the regulation of Polysaccharidase (Carboximetilcelulase [CMCase], xylanase, amylase) activities involved in plant cell wall digestion and activities of glycoside hydrolases (α-l-arabinosidase, β-d-cellobiosidase, β-dglucosidase,β-d-xylosidase) induced by the addition of Sc to the diet [9]. This regulation is mainly the consequence of the impact of Sc on the activity of liquid associated bacteria (LAB) and solid-adherent bacteria (SAB), in cecum and colon. Jouany et al. [9] demonstrated that yeast improves activity of SAB CMCase in cecum and colon as well as β-d-cellobiosidase and β-d-glucosidase in the cecum. Sc also improves xylanase and β-d-xylosidase in the cecum, while it reduces colonic LAB α-amylase activity. In summary, Sc has a positive impact on most enzymes involved in plant cell wall digestion, and as a result it may contribute to explain a better digestion of fiber that has been reported in Sc-supplemented horses.

In this regard, Glade [14] and Pickard and Bertin [15] observed that the addition of Sc to the rations of lactating mares increases milk production. The nutrient density of the milk produced and the transfer of nutrients to the suckling foal and these nutrients are absorbed with high efficiency and retained into body tissues in greater amounts. The biochemical and metabolic effects of Sc supplementation of lactating mares are reflected in more rapid rates of gain by their nursing foals. Sc supplementation can enhance nutrient digestibility and stabilize the caecal and colonic pH, which may contribute to a more stable gut ecosystem, with subsequent improvements in health, lactation and performance of both mares and their suckling offspring [14, 15].

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YEAST ACTIVITY AGAINST PATHOGENIC BACTERIA

Studies performed in other species may help to enlighten this topic in horses, since physiology of most mammals have many similarities. It has been demonstrated that the addition of Sc in the diet of farm animals and even humans, helps to preventing some infectious diseases caused by pathogenic or opportunistic bacteria. Four known mechanisms may help to explain the protective influence of Sc against pathogens in domestic animals and humans. A) Sc exerts a competitive activity for space and sources with other microorganisms; [16] B) Sc has a metabolic influence of on the intestinal ecology that favors lactobacilli; [17] C) Sc is able to adsorb some pathogenic or opportunistic bacteria to its cell wall excluding them from the intestinal ecosystem; [18] and D) Sc is able to regulate the immune system activity through the gut associated lymphoid tissue (GALT). [19] Furthermore, yeasts produce proteolytic enzymes able to degrade bacterial toxins and inhibit bacterial adherence to gastrointestinal epithelial cells through the digestion of cell receptors for certain pathogens such as *Clostridium* difficile [20]. Additionally, Sc is able to induce formation of conglomerates yeast-bacteria by bacterial adhesion to the yeast's cell wall [17, 18, 21].

Previously, it has been mentioned how yeasts are able to influence the intestinal ecosystem favoring the growth of some beneficial microorganisms, which in turn may limit the growth of some pathogenic and opportunistic bacteria. On the other hand, bacteria may be adsorbed to the cell wall of the Sc, due to the interaction of mannose-containing oligosaccharides of the Sc cell wall with the fimbrial lectin of *Escherichia coli*, *Samonella* and other bacteria [17,18]. The adhesion of the bacteria on the Sc cell wall forms conglomerates Sc-bacteria that may limit the interaction of these bacteria with the mucosal cell membrane preventing infection [21].

It has been observed that yeast, as well as other microorganisms, contact cells from the immune system through ileum M cells, which transport microbial cells from intestinal lumen to Peyer's patches interstitium, where the microorganisms are ingested by phagocytosis by macrophages. These cells will destroy the microbial and secrete cytokines to stimulate a strong immune response, promoting the migration of T lymphocytes from blood stream to Peyer's patches [22-26] and stimulatinge the production of IgG and IgA antibodies [6, 27]. The Sc cell wall is rich in beta-glucans, a powerful stimulator of the immune system, and therefore it could be hypothesized that once one Sc is processed by a phagocyte, the beta-glucans of the yeast may be enhancing an immune response against any other microorganism that the same macrophage has engulfed, therefore, enhancing the immune response against other microorganisms.

THE ROLE OF SC AGAINST SOME MYCOTOXINS

Mycotoxins are toxic secondary metabolites produced by fungus that may grow in cereals, silage, forages and among many other food substrates including those for humans, when they are not stored appropriately. These toxins can be cancerigenic, teratogenic, mutagenic, hepatotoxic, immunosupresive, and they can cause damage to brain, kidney, and to reproductive and digestive systems in farm animals and humans.

Table 1. Most relevant Micotoxins and Fungal species producing them

Fungal species	Micotoxin
Aspergillus Flavus and *A Parasiticus*	Aflatoxins
A Ochaceus, Penicillium viridicatum and *P cyclopium*	Ocratoxin A
Fusarium culmorum, F. graminearum and *F poae*	Zearalenone
F culmorum, F. graminearum and *F sporotrichioides*	Deoxinivalenol, vomitoxina
F proliferatum and *F verticillioides*	Fumonisines
Fsporotrichioides and *F poae*	T-2 toxin
F sporotrichioides, F graminearum and *F. poae*	Diacetoxyscirpenol
Acremonium coenophialum	Ergotic alkaloids

Adapted from Denli and Perez (2006).

There are many toxigenic fungal species;, however only a few have been recognized as producers of highly toxigenic mycotoxins. Table 1 lists some of the most studied micotoxins [28]. As it is shown in that Table, some mycotoxins can be produced by more than one fungal species, and more than one mycotoxin could be produced by single speciespecies. If animal - feedstuff is contaminated with mycotoxins, the best way to avoid intoxication would be to discard such material. However, sometimes it is not possible to discard the contaminated feedstuff because of supplies shortage, for economic reasons or simply because mycotoxins may come inadvertently with the feedstuff. Whatever the reason, some preventive measures should be taken in order to avoid animal intoxication.

There are different ways to reduce toxicity of contaminated feedstuff. Destruction of mycotoxins can be achieved by chemical or physical methods. Chemicals include ammonia, hydrogen peroxide, ozone and some acids and alkalis.

However, these methods are expensive are not completely effective. The physical methods include high temperature, UV or X ray or microwave exposure;, however these techniques are little practical and may be too expensive.

A different approach used to reduce mycotoxins in feedstuff is the utilization of mycotoxin-binding adsorbents. Adsorbents can be of inorganic or biological origin. There is a large list of adsorbent compounds such as Silicium dioxide (SIO2), aluminum oxide (Al2O3), iron oxide (Fe2O3), calcium oxide (CaO), Magnesium oxide (MgO), sodium oxide (Na2O), Potassium oxide (K2O), clay, activated charcoal and aluminumsilicates [7]. The main representative of biological adsorbents is the Sc, either in its active form or cell walls. The cell walls harboring polysaccharides (glucan, mannan), proteins, and lipids exhibit numerous different and easily accessible adsorption centers including different adsorption mechanisms, e.g., hydrogen bonding, ionic, or hydrophobic interaction. Therefore, it has been widely used for this purpose [7].

Actually none of the adsorbents is effective for all mycotoxins;, different adsorbents work differentially with each mycotoxin because of the diverse nature of the toxins [7].

To get the most adsorbents, they should be used as mixtures depending on the expected mycotoxins to be removed. Table 2 shows an example of differential mycotoxin removal from feedstuff when adding only yeast cell walls or a mixture of inorganic salts plus yeast [29,30].

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Table 2. Comparison of the use of *Saccharomyces cerevisiae* and a mixture of mycotoxin adsorbents to remove mycotoxins from feedstuff

Micotoxin	AFB1 200 ppb	T-2 300 ppb	OA 400 ppb	Zear 1200 ppb	Don 800ppb	FB1 2ppm
Glucanmannanes	96.22	33.51	25.17	43.75	78.43	89.33
Commercial Adsorbent formula	805	765	3451	4060	4950	N.A.

AFB1, Aflatoxin B1; T-2, T-2 toxin; OA, Ocratoxin A; Zear, Zearalenone; Don, Dioxinivalenol or vomitoxin; FB1, Fumonisin B1; ppb, parts per billion; ppm, parts per million. Adsortion efficiency values are expressed in percentages. N.A., no data available. Adapted from De Luca [28] and Cheminova [30].

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DOSIFICATION

There are many commercial strains of Sc,; each company has made efforts to test their own strain in many different species as probiotic or prebiotic, in animal production. Comparisons among strains are difficult because of methodology differences. However, it seems that each Sc strain keeps some differences with the others. Despite the differences, most strains can be given at a 1-3kg/ton of feed. Otherwise, a more specific dose approach would be to give about 20-30 g a day to foals, 40-60 g to mares and stallions depending on physical activity, and it must be provided mixed or added on top of feed in a continuous dose manner so that the Sc is able to exert all its beneficial effects. It would be wise to observe the recommended doses stated by the commercial brands before using a determined yeast product as a feed additive.

CONCLUSION

Sc is commercially available yeast that has been used for many years in animal nutrition. Yeasts have been found to be very useful to improve nutrition in horses under high -energy demand such as high- performance animals, pregnant mares, rapidly growing yearlings and animals recovering from illness. It has been found useful, also, to prevent several feed-associated disorders, such as starch -associated colic, some gastrointestinal infectious diseases and some mycotoxicosis. Adding Sc to a horse's diet could result in a cost- effective and rewarding nutritional managing practice.

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Chapter 7

UTILIZATION OF ZILPATEROL HYDROCHLORIDE IN FINISHING LAMBS

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ABSTRACT

The β-adrenergic agonist (zilpaterol hydrochloride) improves animal production because it manipulates the efficiency of food use, affecting carcass characteristics and the chemical composition of meat by reducing fat and increasing protein content. This chapter reviews some aspects of the β-adrenergic agonist mechanism of action and chemical structure, growth performance, carcass and meat quality, and meat sensory of lambs under intensive feeding.

INTRODUCTION

The preference of current consumers for lean meat has increased at the same time that fat intake from animal products has considerably decreased; sheep meat has not been the exception; therefore, new feeding techniques have been researched so as to improve productive response, carcass and meat quality, favoring protein accretion and reducing fat, which has brought about investigation about β-adrenergic-agonists (βAA); a group of drugs thatare used in animal production to improve nitrogen retention, known as ‘‘energy deliverers’’ or β-adrenergic-agonists. Their function is at adrenergic receptors level, deriving feed energy into protein synthesis and lipolysis into muscular protein [21]. In theory, the

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utilization of these substances presents a lot of advantages related not only to better animal performance but also to quality since meat from treated animals with βAA have more lean tissue. Information about the use of these substances with ß-agonist activity comes from the eighties, based on previous studies developed in non-obese rodent bio models, utilized clenbuterol as an agent to alter carcass characteristics of steers, observing an increase in muscular mass and a decrease in fat tissue [30]. In Europe, the use of βAA is forbidden in animal production because it is considered as a public health threat [11]; in Mexico, some of them, such as hydrochloride zilpatherol (HCLZ), have been used in sheep and cattle [7, 24 29, 32, 37]; hydrochloride clenbuterol has been used in cattle [12, 33], and ractopamine in pigs [20]. However, the wrong use of clenbuterol has caused risks to public health. Therefore, in Mexico, the Nom-061-Zoo-1999 prohibits its use. This norm does not include ractopamine and HCLZ, which are substances with less potency in broncodilatation, vasoconstriction and cardiac frequency than clenbuterol [36]. South Africa is another country that allows the use of HCLZ in cattle at similar doses (0.15 mg/kg BW/d) to those approved in Mexico [7, 29]. In Mexico, the results of investigations carried out about the use of HCLZ over performance, carcass quality, chemical and sensory attributes of meat have been too variable. That is why the objective of this chapter is to provide information thathas been obtained from the use of HCLZ in finishing sheep fed.

MECHANISM OF ACTION OF βAA

Target receptor of βAA are muscular cells; so far, three subtypes of receptors (β_1, β_2 y β_3) have been identified; in the majority of mammal cells, there have been βAA receptors identified; however, their distribution and proportions vary from one tissue to another in each animal species [21]. The model proposed by Ostrowski [28] and reproduced by Mersmann [21] shows seven transmembranary domains, relatively hydrophobic fixed to the receptor in the plasma membrane. Furthermore, there are four extracellular portions that protrude from the membrane (three buckles connect the adjacent transmembranary domains) and another four intracellular portions at the interior of the membrane (other three buckles connect transmembranary adjacent domains).

The linking union site is the center of the seven transmembranary where aminoacids from several domains are implied. Places of interaction with Gs protein are localized in certain zones of buckles 2, 3 and 4 [28]. In order to avoid indefinite activation of the βAA receptor, either the βAA is eliminated through "reabsorption" or degradation or it is inactivated by other mechanisms. Receptors β_1 and β_2 coexist in the posterior biceps and in the *Longissimus dorsi* area [9]. Therefore, muscular development of posterior quarter, as well as in *Longissimus dorsi* at 12ª-13ª ribs, is bigger in lambs treated with βAA due to an increase of muscular diameter fibers type II, which are known as glycolytic; this causes muscles to be affected specially in these fibers, mainly in posterior quarters and *Longissimus dorsi*. The action mode of a membrane receptor is when the βAA receptor acts in the formation of agonist-receptor complex, with the intervention of protein G, which is a regulator of guanine nucleotides, activating adenilcilase enzyme (AC) and consequently increasing a second intracellular messenger AMPc. The AMPc acts over a secondary protein kinase effecter

(PKA). These last ones change the cellular function and the corresponding effect [10, 21] (Figure 1).

The *invivo* mechanism of a βAA may become complex due to several secondary effects that result from physiological or hormonal response of numerous target tissues. It is difficult to measure a small alteration of the rate of a metabolic function during the minutes or hours that an experiment lasts; a small change *in vivo*, during weeks or months of administration of a βAA, would produce relevant changes in the size of a muscle, a fat deposit or in the whole organ activity [21].

Hydrochloride zilpatherol (HCLZ) is a potent analogue of catecholamine's that acts more frequently on β_2 receptors in smooth muscle, skeletal and adipose tissue, modifying cellular metabolism. It can change cellular metabolism favoring protein synthesis, which is why it is known as a "distributer agent" in cattle feeding [35], being more potent than ractopamine [35]. Urine is the principal way of excretion, but it is also eliminated through feces [38].

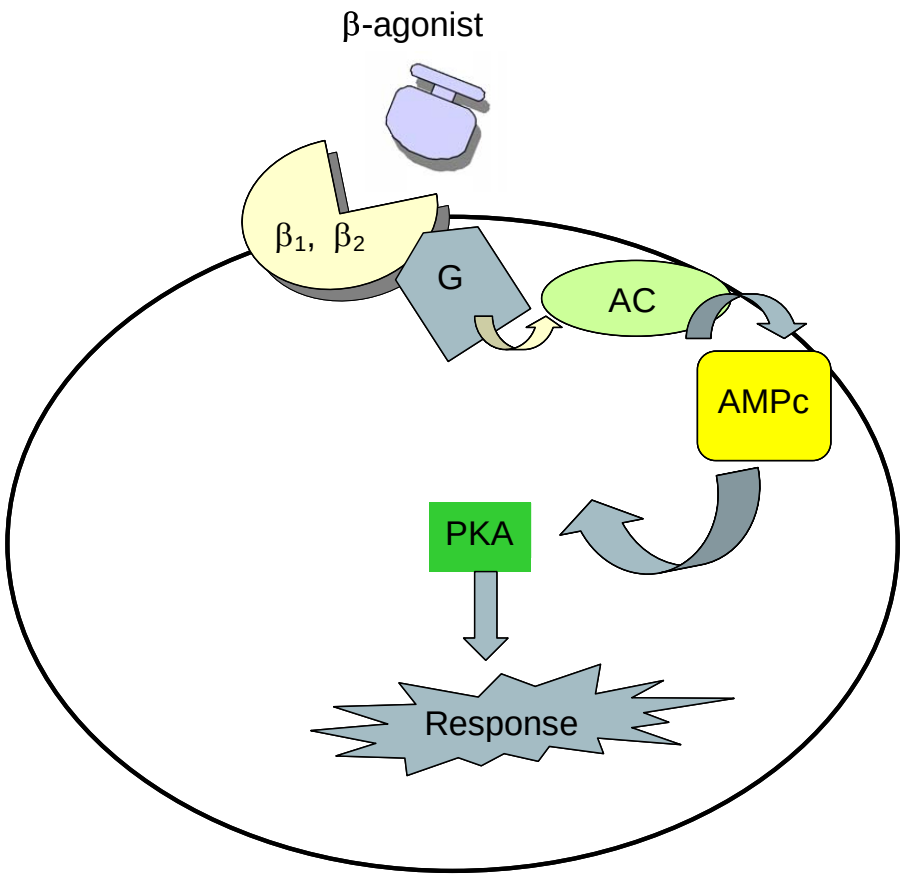


Figure 1. Principal β-adrenergic receptors (β_1, β_2) with their respective transducer system (protein G); their primary effecter (enzyme adenilciclase, AC); second messenger (adenosin cyclic monophosphate, AMPc); and secondary effecter (protein kinase, PKA).

ANIMAL PRODUCTIVE PERFORMANCE

Some investigations agree that HCLZ does not affect the productive response of sheep under different production systems [1, 32]. However, studies carried out in the Faculty of Veterinary Medicine in the University of the State of México show that in increasing HCLZ dose, there was a lineal increase ($P<0.05$) in daily weight gain and a feed conversion decrease in wool sheep [24]. Similarly, in another study, there were differences observed ($P<0.05$) in feed dry matter intake, gaining weight and feed efficiency in favor of hair sheep with HCLZ [4, 31]. Also, in cattle, effects on productive performance with βAA use have been observed [29]. However in other studies, βAA L-644,969 did not improve productive response in sheep [13, 34].

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CARCASS CHARACTERISTICS

Recently, there was reported an improvement in yielding and quality of sheep hair carcass; however, it was not an effect on fat reduction [4]. On the other hand, Mondragon indicated that in wool sheep, perirenal fat decreased linearly ($P<0.05$) when HCLZ was increased [24]; this was similar to Castellanos working with cattle [7]. However, the use of βAA L-644,969 did not affect fat carcass deposition in wool sheep [34].

Literature shows that in sheep, response to βAA is not clear [34], and it was confirmed that effects of βAA on fat tissue are not persistent as in muscular tissue, and in some cases it results incontradictions. Mondragón found a cubic effect ($P<0.05$) on increase of *Longissimus dorsi* area (20. 4vs. 30.1 cm^2) and a decrease of dorsal fat at sixth rib (1.0 vs. 0.2 mm) with a dose of 15.9 mg HCLZ/kg DM, and an increase of perimeter leg (37.2 vs. 41.7 cm) with a dose of 5.3 mg HCLZ/kg DM in wool sheep [24], similar to what was found by Salinas whose study indicated that *Longissimus dorsi* area increased (9.8 vs. 14.3 cm^2) with a dose of 6.0 mg HCLZ/kg DM in hair sheep [31]. Similar results were found by Shackelford, with a dose of 1.0 mg L-644,969/kg DM in wool sheep [34]. Also, they reported a linear effect ($P<0.05$) on bigger and minor thorax width when HCLZ dose was increased in wool sheep [24]; similar results were found in Rambouillet lambs by Valenzuela [37] and in cattle by Castellanos [7]. The findings may concern to β_1 and β_2 adrenergic receptors thatcoexist in posterior biceps and *Longissimusdorsi* eye area, whichis why these muscles are specially affected [9, 14]. In contrast, Salinas mention that *Longissimus dorsi* eye area was not affected (6.53 vs. 6.24 cm^2) when 10 mg HCLZ/kg DM were used in Pelibuey lambs [32], and Koohmaraie indicated that there was not effect (17.8 vs. 19.1 cm^2) in rib eye area when 4 mg L-644,969/kg DM were used in Dorset x Romanov sheep [13].

Beermann pointed out that bone and carcass length were not affected when treated with βAA [6]. These findings are similar to results obtained by Mondragon[24]. However, the use of βAA L-644,969 in cattle did not affect the carcass length [22]. Mondragon found that hot (19.4 vs. 20.9 kg) and cold (19.4 vs. 20.9 kg) carcassweight increased linearly when a dose of 15.9 mg HCLZ/kg DM was used in sheep [24]; similar results were obtained by Valenzuela in lambs with 15 mg HCLZ/kg DM; carcasses showed a bigger weight and yield [37].

Nonetheless, the last results are different fromwhat was reported by Shackelford about hot carcass (30.6 vs. 32.5 kg), cold carcass (29.5 vs. 30.4 kg) weight, and dorsal fat thickness (4.4 vs.3.7 mm) [34].

MEAT QUALITY

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Mondragon pointed out that intramuscular fat content of *Longissimus dorsi* linearly decreased (48.9 vs. 41.1 g/kg) and crude protein (CP) increased (168.7 vs.189.1) as the dose of HCLZ was increased to 15.9 mg HCLZ/kg DM in Rambouillet lamb diet [24]. This was similar to other reports where βAA increased muscle protein retention [19, 22]. However, the last results are different fromKoohmaraie findings, which used L-644,969 in sheep and did not observe an increase in protein retention in meat [13). Koohmaraie and Beermann indicated that the intramuscular fat lowering by using βAA is in a lesser grade [6, 13]. Nonetheless, βAA (L-644,969) decreased fat depots in *Longissimusdorsi* (3.0 vs.2.2 %) in cattle [22]. HCLZ also tends to increase water content in carcass [30], and then muscle has more humidity [11]. This influences quality, juiciness, softness, color and flavor of meat [25]; in contrast, Moloney indicated that humidity was not affected when L-644,969 (73.3 vs.73.9 %) was used in cattle [22] or in Dorset x Romanov sheep (56.9 vs.58.1 %) [13].

Some studies have shown a lower tenderness of meat when HCLZ is used; with a dose of HCLZ of 15.9 mg/kg DM, there was an increase of the cut force (2.3 kg F/cm^2) in *Longissimus dorsi*; [24].

These values are within what is recommended by USA and Australia markets and are smaller than what was reported by Koohmaraie, 20 days postmortem in sheep (8.2 vs 10.9 kg) [13], and by Avendaño, five days postmortem in cattle (4.39 vs 5.11 kg) [4].

Regarding this, Koohmaraie mentioned that by adding a βAA in sheep diet, there was a lesser protein degradation at muscular level during the first 20 days postmortem; this is due to the fact that calpastatins inhibit proteases activity [13], which impedes muscular protein degradation, giving as a result a tougher meat. Nonetheless, calpastatins are sensible to freezing, and their activity decreases during the process. On the other hand, calpastatins are not destroyed and retain their activity after freezing. It is possible that very low activity continues during freezing, giving place to a certain level of proteolysis, and so, an improvement in meat softness [8].

SENSORY ATTRIBUTES OF MEAT

The sensorial analysis of sheep meat treated with HCLZ has not been studied enough in Mexico. However, Mondragon indicated that the use of different doses of HCLZ in sheep did not affect ($P<0.05$) satisfaction grade of consumers [23]. This means that consumers showed an acceptable satisfaction grade when they ate sheep meat treated with HCLZ. Likewise, in an study carried out in the USA in swine meat treated with ractopamine using a dose of 10 ppm during 28 days, it did not affect ($P>0.05$) palatability of muscle *Longissimus*. However, in another study performed with cattle in that country, HCLZ modified sensory attributes like juiciness, tenderness, flavor and color of meat [17].

In finishing pigs, Mariezcurrena observed that the use of ractopamine during the last 28 days increased intramuscular fat, and when protein and lysine were reduced, juiciness of meat also increased, according panelist criteria [20]. On the other hand, Apple found that diets with ractopamine and a high concentration of lysine (3.1 g/Mcal) reduced intramuscular fat, as well as increased the cut force of swine meat [2]. Kutzler pointed out that in pig diets with 17.7% crude protein and 3 g of lysine/ Mcal, the ractopamine linearly decreased marbling and intramuscular fat in *longissimus dorsi* [16].

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CONCLUSION

Certain βAA do not induce the same effect in all livestock species. Results are variable, because probably the receptors do not become completely active; also, it could be that receptors become inactive very fast or thatsome animal species have a limited number of receptors, which would decrease a normal response. For example, the effects observed with HCLZ in sheep have caused positive changes in weight gain andfeed efficiency without modifying DM feed intake. In other studies, there has only been reported good carcass yielding but without improving meat texture and fat deposition.

Nonetheless, results obtained can vary according to the dose of βAA, diet used, age, sex, and animal breed, as well as environmental factors. Because of these variations, effects observed after administration of a βAA like HCLZ are complex and hard to explain.

Therefore, on one hand, it is recommended to carry out investigation to recognize factors that cause this variation at a physiological level in animal (health and animal welfare), as well as the effects of βAA in physiology and human health, so that it can allow supporting the use of βAA or its elimination from animal feeding.

However, on the other hand, because of cases of human intoxication caused by consumption of meat with βAA, and considering ethic principles and animal welfare, the use of these products should be under strict norms of security when they are used in primary production or under experimental goals, which would contribute to improve basic health, biological animal function and would allow animals to live as they are genetically programmed; in this way, there will be more probabilities to assure quality and innocuous meat production, without risks to the final consumer.

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Chapter 8

USE OF ZILPATEROL HYDROCHLORIDE IN SHEEP FEEDING

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ABSTRACT

The present chapter constitutes a review of β-adrenergic agonists (βAA) in sheep production, focused on the effects of productive performance and carcass characteristics of different breeds, who were given two drugs (zilpaterol hydrochloride; ZH and L-644, 969) in different doses. Some results on weight gain, food intake and efficiency, in terms of productive performance, are presented. For carcass characteristics, results highlight the performance of whole carcass, cuts, length, shape, ribeye area, fat percentage, and instrumental meat quality. The results show an inconsistently positive effect on improving animal performance, even in most of the carcass characteristics suggesting that more studies should be conducted. The effect of β-adrenergic will depend, among other factors, of age, gender, environment, nutrition, and application time. It is always advisable to assess the cost-benefit ratio when including this type of β-agonists in animal feed.

INTRODUCTION

The β-adrenergic agonists (βAA) are organic molecules steroids [22], belonging to the catecholamines [28]. They are chemically synthesized substances that are linked to β-adrenergic receptors that mediate cellular and physiological natural, therefore, seeming to

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noradrenalin (norepinephrine) and adrenalin (epinephrine) not only in its mechanism of action but also in its chemical structure as it is structural analogues of β-phenyl-ethanolamine (arylethanolamine). One option to improve production efficiency is the use of βAA [16; 24] as the zilpaterol hydrochloride (ZH) 3; 17; 24] or L-644, 969 [11; 13; 25] in sheep.

South Africa, USA and Mexico allows the use of βAA ZH (ZILMAX, Intervet) in animal feed [21], mainly in beef cattle, achieving favorable results for this species, with established doses of 0.15 mg/kg BW day [7; 22]. Zilpaterol hydrochloride acts preferentially on β2 receptors in smooth muscle, skeletal and adipose tissue by modifying cellular metabolism. It can change the cellular metabolism for protein synthesis, by what is known as a "delivery agent" in the diet of beef cattle [28], being more potent than Ractopamine [26]. Its main route of excretion is in urine and feces [28]. In fat metabolism, βAA acts indirectly on fat deposition by increasing the metabolic rate and energy expenditure of animals and has a direct effect on increased levels of adenosine monophosphate cyclic (AMPc) in adipose tissue [14]. In lipolysis, the body fat is transformed into glycerol and fatty acids; the latter enter into the circulation and are distributed throughout the body, in the cells are degraded by β-oxidation to acetyl-CoA, which enters the Krebs cycle for energy production. Therefore, lipolysis decreased the synthesis of fatty acids and triglycerides, resulting in less accumulation of fat in the body [27]. Also there is improved carcass composition by reducing fat accumulation and consequently there is a higher percentage of muscle [9; 28]. The protein metabolism in animals treated with βAA increases protein retention [13], even though there must be sufficient amino acids available [7]. Muscle hypertrophy seen in the "target muscle" that is more abundant in the leg than in other regions. In sheep and cattle, it has been observed to increase the muscles weight up to 40%, and magnitude of the response varies depending on the βAA studied and the influence of factors such as species, age, sex, diet and breed [14]. In muscle hypertrophy, it includes changes in the type of muscle fibers [15]. They have also shown to have an effect by increasing the water content in the carcass [23]; as a result, the muscle has more moisture.

PRODUCTIVE PERFORMANCE AND CARCASS CHARACTERISTICS

The use of β-adrenergic L-644, 969 as growth promoter in sheep increases growth rate, efficiency of feed utilization, carcass weight, weight of *biceps femoris* muscle and ribeye area [8]. In crossbreed animals, vulnerable to stressful environments, L-644, 969 increased the weight of biceps femoris and ribeye area, but decreased the fatback thickness [13].

Koohmaraie et al. [11] confirm that use of L-644, 969 improves meat characteristics. Ewes and lambs fed with L-644, 969, clenbuterol and ractopamine, respectively, increased the proportion of transcription in muscles and increased the synthesis of ribonucleic acid messenger (RNAm) in muscle fibers [4]. In studies with sheep fed ZH for 30 days, there was a significant increased carcass performance ($P \leq 0.01$) from 55.0 ± 1.9% to 56.7 ± 1.75% in 45kg live weight (LW) animals and 57.9 ± 1.7% to 59.1 ± 1.8% in 50 kg LW sheep [5]. Likewise, heavier animals had better conformation and increased carcass length ($P \leq 0.05$) than lighter [6]. Moreover, feeding ZH has increased ($P \leq 0.01$) ribeye area from 16.3 ± 3.1 to 18.4 ± 2.9 cm^2 in 45kg lambs [6] and 17.8 ± 2.4 to 21.1 ± 2.1 cm^2 in 50kg sheep [6]. Similarly, ZH has led an increment in muscle percentage from 61.7 ± 2.3 to 66.0 ± 2.4% in

carcass of animals slaughtered at 45 kg [6] and 60.8 ± 2.7 to 65.6 ± 2.5% in carcasses of sheep slaughtered at 50 kg [6] with a reduction in the amount of fat from 11.5 ± 1.9 to 9.2 ± 2.3% in 45kg sheep and 13.31 ± 3.2 to 10.1 ± 2.7% in carcasses from sheep slaughtered at 50 kg. All this indicates that use ZH at dose of 0.15 mg/kg LW improves carcass properties in sheep [6].

In this sense, Mondragon et al. [18] indicated that use of ZH in sheep increased water content, protein and hardness in *Longissimus dorsi* muscle. Supplementation of 15.9 mg/kg DM of ZH in the diet showed positive effects on growth and carcass characteristics. Avendaño et al. [2] added 10 mg ZH/d to the diet of hair breeds lambs, observing hot carcass weight (17.2 vs. 14.9), performance (53.8 vs. 46.0), conformation (7 vs. 6 units) and ribeye area (18.9 vs. 15.5 cm^2) with higher results ($P < 0.05$) in the treated group with ZH, compared with the control group. In relation to yield production in meat cuts, adding ZH improved ($P < 0.05$) percentages of legs (24.5 vs. 23.9 %), ribeye area (12.9 vs. 9.9%) and hindquarters (48.3 vs. 46.6%). The data indicate a positive effect on carcass characteristics and percentage of some meat cuts, even under heat stress [2].

However, in different studies carry out in sheep including ZH in the diet at different doses, there were not differences ($P > 0.05$) in the productive response in Pelibuey sheep [1; 12; 24] with daily weight gains from 240 to 323 g/d and average feed conversion of 4:1. Likewise, Koohmaraie et al. [11] and Shackelford et al. [25] indicated that there were not differences ($P > 0.05$) in the daily weight gain and final weight using L-644, 969 in the diet in Dorset x Romanov sheep and Rambouillet lambs, respectively.

Casaya and Partida [4] observed that use ZH for 30 days prior to slaughter of sheep decreased daily weight gains of animals who received it in about 20%, diminished from 322 to 260 g in lambs Kathadin x Charolais and 350 to 276 g in Kathadin x Dorper lambs. Other studies in sheep fed ZH report that the average behaviour (weight gain, feed conversion) did not improve animal production [19]. In contrast, Aviles and Pulido [3] mentioned the use of ZH affected ($P < 0.05$) drip loss and backfat in carcass and tenderness in meat (5.5 vs. 4.8 points); that is, the meat of sheep supplemented with ZH was softer. The data obtained by Moreno et al. [19] indicate that most of the parameters characterizing the carcass were not influenced by inclusion of ZH in the diet, so it was not helpful for improving meat qualities. Only significant differences ($P \leq 0.02$) in the cold carcass weight (19.8 ± 2.9 vs. 22.3 ± 2.9), thorax width (21.4 ± 2.1 vs. 23, 9 ± 2.9) and chest circumference (24.5 ± 2.0 vs. 27.9 ± 3.9) for diets without and with 6 mg / kg ZH, respectively, were found. Besides the quality instrumental variables (texture, pH, water holding capacity and color) of meat were not modified by the inclusion of ZH in the diet of animals, only the *Longissimus dorsi* muscle area was affected ($P \leq 0.01$) by the inclusion of ZH in the diet (13.37 *vs.* 15.82 cm^2).

CONCLUSION

The use of ZH and L-644, 969 in diets for sheep shows no consistent effect positive on improving animal performance or most of the carcass characteristics, which suggests that there should be more studies on this item. The effect of β-adrenergic receptors will depend among other factors on age, gender, environment, nutrition, doses and time of application. It

is always advisable to assess the cost-benefit ratio when including this type of β-agonists in animal feed.

Table 1. Effect of zilpaterol hydrochloride (ZH) and L 644.969 on the sheep productive performance and carcass characteristics

βAA /(dose)	Productive response	Carcass effect	Breed	Author
L-644,969		Reduced meat tenderness	Dorset	[11]
L- 644,969	No increased in weight gain neither final weight		Dorset x Romanov and Rambouillet	[11] [25]
L- 644,969		Increased weight of the biceps femoris and ribeye area, but decreased backfat thickness		[13]
L- 644,969	Increased growth rate and feed efficiency	Increased carcass weight		[8]
L- 644,969		Increased transcription and synthesis (RNAm) in muscle fibers		[4]
ZH (10mg/kg)	There were not differences		Pelibuey	[1] [12]
ZH (125mg/100 kg)	There were not differences	There were differences (W-B shear force) in chops, fat deposition and depth of chest	Suffolk Rambouillet	[3]
ZH (15,9 mg/kg)	There were not differences	Increased water content, protein and *Longissimus dorsi* muscle hardness.	Rambouillet	[18]
ZH (10 mg/d)		Indicated a positive effect on carcass characteristics and percentage of some cuts, (under conditions of heat stress)	Hair sheep	[2]
ZH (0,15mg/kg)	Reduced weight daily gain about 20%	Raised carcass yields; increased size of the steak, percentage of muscle, reduced amount of fat	Kathadin x Dorper, Kathadin x Charolais	[5]
ZH (0,15mg/kg)		The carcass characteristics were not influenced neither instrumental meat quality	Pelibuey x Blackbelly and Pelibuey x Dorset	[19]

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Chapter 9

SELENIUM SUPPLEMENTATION AND THE IMMUNE RESPONSE OF SHEEP

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ABSTRACT

Selenium is one of the trace minerals that is essential for animals; it is a necessary for a suitable contribution of this element in the diet to maintain their health and productivity in general. Most of the selenium is content in erythrocytes as a component of glutathion peroxidase (GSH-Px), an enzyme that plays an important role in cellular processes of oxide reduction, to assume an important mechanism of cellular defense against forms of highly reactive oxygen (radical free) that occur in the body during normal aerobic metabolism. This work considers the importance of selenium in animal metabolism, the determinants for the presentation of pathologies associated with this micronutrient deficiency and its significance of supplementation for a proper activity of the immune system in sheep. The minimum requirement of selenium for pets varies according to ingested selenium compounds, the criteria of adequacy used and the nature of the rest of the diet, particularly vitamin E content. The daily requirement of selenium required for the metabolism of this mineral is carried out fully and effectively is satisfied with a level of 0.3 ppm in the dry matter of the ration. Before starting a supplementation program, however, it is necessary to consider the factors that modify the metabolism of selenium (as: chemical form of the mineral, pre-existing blood and tissue concentration, interaction with other minerals and calcium concentration in the ration). The various manifestations of selenium deficiency can be prevented by adding it in rations as food supplement in the form of salts, periodic injections or treatment of the soil to increase the concentrations of grasses to satisfactory levels. The method of choice will depend on the

conditions of management and need to minimize the quantities of selenium introduced in the soil-plant-animal-man cycle.

INTRODUCTION

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Selenium acts upon the development of animals and their production, and therefore it has been important that the soil-plant-animal system be evaluated since it has been observed that soils with deficiencies in this micronutrient have low availability for the plants and forages that grow in the sheep grazing areas, with obvious consequences in these animals [37].

Selenium concentration and bioavailability in soil is influenced by the interaction of different factors, and the concentrations considered to be appropriate are 0.1 to 0.5 ppm and deficiency levels to be 0.024 to 0.096 ppm. In the case of forages and grains, the appropriate levels are 0.1 ppm; from 0.075 to 0.1 ppm, they are considered as moderate and from 0.05 to 0.075 as low and 0.05 ppm as deficient [3]. Low selenium levels in the cycle soil-plant-animal directly affect the latter with clinical alterations and manifestations of nutritional myopathy, which has become a problem in different regions, reflecting low organic levels in sheep [8, 48, 52]. The main biological role of selenium is to form part of the glutathion peroxidase (GSH-Px), which contains four selenium atoms per mol of enzyme. The function of this mineral is to participate in cell protection, destroying oxidizing agents that are products of the organic metabolism [2, 3, 45]. Most of the amount of GSH-Px is found in erythrocytes, and the development of these cells, rate of erythropoiesis and their elimination depends on selenium use. The reported values of GSH-Px under controlled supplementation conditions have been considered deficient when they are 245 U/g Hb and high when they are 554 U/g Hb [12]. Tissue levels found in lambs ages 0 to 90 days indicate that the most drastically affected age is between 31 to 45 days, suggesting a congenital and late presentation of nutritional myopathy; therefore availability of selenium in grazing areas for the dams is deficient, causing in the lambs progressive cachexy, locomotion problems, and it has also been observed that selenium-deficient lambs show immunosuppression, reducing the humoral immune response of serum IgG concentrations, becoming more susceptible to pathological processes [46, 52]. Based upon the diagnosis of selenium blood levels in sheep, values below 0.05 mg/kg are considered to be deficient; from 0.05 to 0.075 mg/kg marginally low; from 0.076 to 0.1 mg/ kg as marginal and above 0.1 mg/kg appropriate [53]. Reports from several studies suggest that selenium may be responsible for the alteration of the immune response, reducing the response capability of the organism to face antigenic challenges due to the depressive effect upon the synthesis of neutralizing antibodies. Therefore, selenium deficiencies compromise immunity, and when there is selenium and vitamin E supplementation, an increase in the immunological response is observed measured by the concentration of serum IgG and hemagglutination inhibition titers in sheep blood [4, 11, 28]. It has been shown that lambs that have low blood selenium levels, with low Glutathion Peroxidase activity, have a low levels of immunoglobulins, low phagocyte activity and higher macrophage destruction. In canines, there is a poor response to Phytohemagglutinin (PHA), Concanavalin A (Con A), Mitogenic response and Streptolysin O (PWM). In contrast, in swine supplemented with vitamin E and selenium, lymphocytic response to Phytohemagglutinin is increased [27]. Low antibody titers in selenium-deficient lambs derive from a low blood GSH-Px activity, increasing lymphoid cell destruction due to

high levels of circulating peroxides [11, 14]. During young ages, milk levels of selenium may cover the lamb's basic requirements. Nevertheless, the amount available of selenium through milk may be reduced with the increase of needs due to factors such as season of birth, high growth rate, higher muscular activity, low or nil supplementation with selenium and/or vitamin E, adverse climatic conditions and high environmental microbial content [36, 43, 53].

A subcutaneous injection of barium selenite has been recommended to treat selenium deficiency, being an effective method used during long periods, since it insures the administration of soluble selenium salts. When barium selenite is given to gestating ewes, satisfactory levels of selenium are achieved not only in the dams, but there is also transference of these levels of selenium to the lambs. Therefore, the use of barium selenite before or during pregnancy of the ewes protects them effectively against selenium deficiency disorders, and the greatest benefits have been experimentally observed in herds maintained with optimal levels of selenium in the diets, with effects on the reduction of mastitis incidence and prevention of losses due to myopathy and respiratory diseases [11, 21, 33].

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IMMUNITY

Humoral immunity is mediated by molecules with antigen specificity, and antibodies are produced away from their site of action. Humoral immunity is easily transmitted by serum, and by cells, humoral immunity is transmitted with more difficulty; the inverse happens in cell-mediated responses [6].

In cell-mediated immunity, cells that have been specifically sensitized are the ones that intervene, coming into contact with the antigen, and they act by direct cytotoxicity or liberation of antigen non-specific mediators. Active immunity in lambs is complicated due to the variations in amount and quality of colostrum that is ingested during the first hours of life and also the absorption of antibodies that have a strong influence on active antibody production [5, 6]. Immunoglobulins are high molecular weight serum proteins, with five classes of antibodies that have independent functions, structure and antigenicity. Serum immunoglobulin classes that have been recognized in sheep are as follows: IgG (IgG_1 and IgG_2), IgA (serum and secretor) and IgM, present in blood and most of the secretions, with relative concentrations varying according to the fluid that is examined [31].

Differences in absorption between immunoglobulin classes have indicated that IgG is absorbed only 90%, IgM 59%, while IgA is absorbed 48%; likewise, there is importance in the time in which such absorption happens. Therefore, IgG is the type of antibody that is found in higher concentration in serum. Due to its small size, it easily escapes from the circulatory flow, into the acute inflammation sites. IgG participates efficaciously in agglutination, opsonization and precipitation reactions with diverse antigens, while it also activates the complement system [31]. Antigenic differences have been shown by immunodiffusion studies specifically between IgG_1 and IgG_2 from serum as well as colostrum. IgG_1 is the one found mainly in colostrum and to lesser degree IgG_2, IgM and IgA. Among these, IgG_1 is the only one that persists up to 60 days postpartum, while the others persist only 12 days [13, 20]. Selenium deficiencies impair immunity, and when selenium and vitamin E are supplemented, there is an increase in the humoral response mediated by antibodies (Ig) and a cellular response mediated by T cells and macrophages [20, 29, 35, 47].

Phagocytic cells, such as neutrophils, and macrophages of animals deficient in selenium have demonstrated reduced amounts of GSH-Px that also have lower amounts of activity and microbicidal action [7, 44].

Selenium and vitamin E deficiencies predispose the animal to having low GSH-Px activity and therefore a reduction in immunoglobulin levels, low phagocyte activity and greater destruction of macrophages. Therefore, it is assumed that the immune system requires appropriate levels of selenium for optimal functioning, since when there are deficiencies in the diet, these negatively affect the immune response with the observation of low levels of antibodies in response to vaccinations, and when selenium is applied it potentializes the protective effect [10, 49].

Besides the GSH-Px activity reduction in neutrophils, it has been reported that its absence causes depression of phagocyte and microbicidal activity of neutrophils and that low antibody titers in selenium deficient lambs derive in poor GSH-Px activity in blood, increasing the destruction of lymphoid cells due to high levels of circulating peroxides [25, 28].

Protection of cell membranes and organelles against oxidative harm given by GSH-Px happens by the effect of the oxygen radicals, when detoxification of lipid peroxides produced during oxidative metabolism occurs, reducing them to non-toxic hydroxyl fatty acids, while vitamin E avoids the formation of fatty hydroperoxides. This effect is of great relevance for the mitogenesis response of lymphocytes and function of macrophages, since in the stimulation process of lymphocytes, the mitogens attack the appropriate receptors of lymphocytes and begin a series of metabolic events, which are greatly associated with the increase of activity of certain enzymatic systems [50]. Hydrogen peroxides, substrates of GSH-Px, act through the myeloperoxidase to aid in the destruction of particles ingested within the neutrophil. GSH-Px may also have a role in the regulation of flow of arachidonic acid metabolism intermediaries through lipoxygenase and cyclooxygenase [50, 51].

During the phagocytosis process in the blood system, there is an important increase in oxidative metabolism, when foreign particles are consumed, with the production of large amounts of hydrogen peroxides and oxygen that are formed during the biological oxidation processes, such as the phagocyte oxidative burst and the formation of lipid peroxides during the chain reaction of free radicals; these peroxides are the substrates of GSH-Px as bactericidal agent [38]. Lipid hydroperoxides are produced during neutrophil phagocytosis of neutrophils and are known to inhibit 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase. Both enzymes are involved in the production of NADPH used in the oxidative destruction of particles ingested by neutrophils [40].

Phytohemagglutinin activation (PHA) of macrophages is associated with the beginning of large vacuoles, suggesting a severe harm to intracellular organelle organization. These alterations are associated with the intracellular production of peroxide, affecting cellular GSH-Px activity due to selenium deficiency, producing cell damage that is reflected in extracellular function reduction with hydrogen peroxides liberation [16, 17, 18]. Reactive oxygen production by phagocytes contributes to the microbicidal activity and the inhibiting substances produced during phagocytosis are lipid hydroperoxides, ancones, superoxides, simple oxygen, hydrogen peroxides, hydroxyl radicals and prostaglandins, which may escape from the cell from the phagosome towards the cytoplasm; this escape may be the potential cause of harm to the phagocytic cells and their functions. Increase in chronic inflammatory activity, produced by oxidative agents, harms lymphocytes and therefore compromises mitogenesis, alters the function of macrophages and other defense cells when there are low

selenium and GSH-Px levels. These data provide support to the role that selenium has in the immune and inflammatory processes [17, 18]. Low GSH-Px activity predisposes to having low immunoglobulin levels, low phagocytic activity and greater macrophage destruction. Harm produced by toxic oxygen induces a reduction in phagocyte activity of polymorphonuclear cells when the diet is low in selenium [39, 41].

Experimentally, lambs from dams that received different doses of selenium and vitamin E supplementation were inoculated with Parainfluenza 3 virus, *Clostridium perfringens* and *Corynebacterium pseudotuberculosis*; the treated animals had higher antibody titers than the control, non-treated animals [28].

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SELENIUM

Selenium as an essential micronutrient associated with vitamin E has an effect on several functions of the animal, such as alteration of reproduction. Moreover, it affects the nervous, muscular, skeletal, hematopoietic and immune systems in their basic characteristics, and it influences the integrity of all the cells of the organism [41].

The biochemical function of selenium is to act as a structural component of the Glutathion Peroxidase enzyme (GSH-Px), and the precise function of this enzyme is to participate in cellular protection destroying oxidative agents such as hydrogen peroxides and lipid peroxides that are capable of causing irreversible denaturalization of essential cell proteins, which in turn causes degeneration and necrosis. This interdependency of GSH-Px activity with the presence of selenium may explain the interrelationship among selenium, vitamin E and sulfur-containing amino acids that may be precursors of glutathione, which at the same time acts as substrate for GSH-Px and the maintenance of sulfhydryl groups in the cells, acting synergically to protect tissues against oxidative harm [2, 24].

It is evident that GSH-Px, which depends on the presence of selenium in the diet, has an important role in detoxification of lipid peroxides by reducing them into non-toxic hydroxyl fatty acids, and vitamin E inhibits the transformation of hydroperoxide fatty acids. When there is a selenium deficiency, plasma levels of peroxides rapidly increase, causing harm to capillary endothelium, red blood cells, plasma cells and muscle fibers amongst others, modifying the cellular homeostatic status harming the metabolic activity of the membrane, which is related with a reduction in antibody titers, causing immunosuppression. Thus selenium deficiencies significantly reduce humoral immune response and the non-specific immune function [1, 10, 12, 40]. Several studies that have been reported that selenium may be responsible for the alteration of the immune response, reducing the capacity of animals to respond to antigenic challenges due to the depressive effect in the synthesis of neutralizing antibodies [30].

SELENIUM METABOLISM

Differences between organic and inorganic nutrients in relation to maintenance and physical work needs derive from the different metabolic destinations of these nutrients. Organic nutrients enter body metabolism from where they emerge as final metabolism

products, in a simpler form without capability of serving as an energy source, as these forms are excreted through the normal pathways. On the other hand, inorganic ions that are liberated in the course of metabolism do not change, nor are they turned into products non-usable by the tissues. They are useful for the reformation of functional combinations as it happens with inorganic ions absorbed by the digestive system. Said reutilization of minerals by the tissues could theoretically be total, determining that the maintenance needs for said nutrients could be nil. Nevertheless, preservation processes of the organism are not as efficient as that, and there is an inevitable filtration of minerals through the kidneys, intestinal mucosa, digestive glands and skin [14, 24]. The capability of the animal organism to preserve minerals that are to be reused in metabolic processes observably differs with the different elements. Selenium as an important reducer of the lipid peroxidation is incorporated within the sulfide contained in the essential amino acids cysteine and methionine, and it is incorporated as selenocysteine in selenoprotein. Assays with selenium may not be representative of the functional selenium status within the animal since the inorganic selenium (selenomethionine) is not specifically incorporated within the protein, and it is also not within the seleno–dependent GSH-Px [19, 46, 48].

Inorganic selenium (sodium selenite) is incorporated exclusively within the selenoproteins, only within a specific amino acid: cysteine, due to the substitution of selenium by a residual cysteine atom of the protein. Nevertheless, organic selenium may be present as selenomethionine and be incorporated within all proteins as a methionine substitute. Selenium consumption dramatically influences the activity of GSH-Px of the body components and also the relative distribution of the enzyme [42].

SELENIUM SUPPLEMENTATION

Diverse manifestations of selenium deficiency may be prevented through the addition of selenium incorporated into mineral supplements or mixed rations, periodical injections or oral doses of selenium salts, the use of licking salt blocks that contain selenium, or the treatment of soil with selenium compounds to increase concentrations in grasses up to satisfactory levels [22, 26, 34]. The chosen method shall depend on the handling conditions and the need to reduce to the minimum the amounts of selenium introduced into the cycle: soil-plant-animal-man [8, 12, 14, 23].

Recommendations for selenium supplementation in several countries vary according to the characteristics of the zones, and therefore there is a range from 1 to 5 mg/ml, dosing at 1 ml/ 100 kg of body weight [22]. The most common procedures to avoid the presentation of ailments that respond to selenium supplementation in animals fed on native grasses are direct subcutaneous injections, generally with sodium selenite or a 1 to 5 mg oral dose per sheep, with this compound at three-month intervals after the first application [8, 14]. Higher repeated doses applied intramuscularly may increase blood levels of selenium in treated ewes [15]. Whenever there are different zootechnical purposes for the animals, oral doses treatments or injections may be given frequently. Injections or oral doses of selenium in the prescribed amounts do not cause excessive or dangerous concentrations in edible tissues of the treated animals [9].

In certain cases of selenium supplementation, significant increases in growth and wool yield have been achieved. Experimentally, in five-month-old lambs that received orally 5 mg of selenium as selenite and that were evaluated two to six weeks after that, there was a reduction of mortality of 27 to 28% as a result of the treatment, and highly significant weight gains were observed. Therefore, production increase determined by selenium treatment is based upon the change in live total weight, including mortality as total loss. Likewise, it has been concluded that the appropriate oral doses are of less than 1 mg of selenium in the first application and 1-5 mg of selenium with three-month intervals after the first treatment [32, 36]. Salt with vestigial minerals, reinforced with sodium selenite in the amount of 26 mg of Se/kg, offered *ad libitum* to ewes and lambs reduces the incidence of ailments, without increasing tissue concentrations above the amounts naturally found in lambs that do not have supplementation and come from different zones [14, 23, 25].

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Chapter 10

ROLE OF SELENIUM IN SHEEP FEEDING: ANTIOXIDANT EFFECT AND REPERCUSSION IN MEAT SHELF- LIFE

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ABSTRACT

In recent years, an increase in the non-transmissible diseases has appeared at the worldwide level as a reason why it is necessary to consume healthier foods; besides having a suitable nutrimental value they provide physiological benefits to the human being. Itis for that reason that recently the denominated functional foods exist in the market. This chapter analyzes selenium as an essential trace element and its role as part of the enzyme glutathion peroxidase, which when present being in a food like meat, protects it against oxidation stress. Also, it is analyzed the possibility that selenium modifies in a positive way the characteristics of the meat during the shelf life.

INTRODUCTION

The consumers in the last years have shown a noticeable interest towards certain foods, and the fact that besides basic nutritious content, they provide benefits to physiological functions. A "*functional food*" refers those that have a specific function in the human organism providing benefits [21]. Selenium is an essential element in the animal diet and is

part of the enzyme glutathione peroxidase (GSH-Px) that provides a defense against oxidation stress, protecting the cellular membranes when acting like antioxidant [17]. There are studies in which it is mentioned that the use of the selenium modifies in a positive way the characteristics of the meat during the shelf life. It can be administered in animal diets in organic form from yeast or like sodium selenite; nevertheless, it is necessary for investigation on the matter that allows a deeper knowledge of its use and benefits.

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FUNCTIONAL FOODS

The objective sought in the antiquity about feeding was that this was balanced, varied and pleasing to the consumers. Today, the focus has been its influence in health, exerting a control and modulation of some aspects of metabolism, of digestive, endocrine, immune and nervous systems, among others, in order to avoid or to prevent some diseases like the non-transmissible ones—or chronicles—that are long-term conditions with a generally slow progression. The four main types of non-transmissible diseases are: the cardiovascular diseases (for example, the myocardium infarcts or cerebrovascular accidents); cancer; the chronic respiratory diseases (for example, the chronic obstructive pulmonary or asthma); and diabetes. Non-transmissible diseases, or NTD, represent different causes of death more important in the world, because they capture 63% of the total number of annual deaths. The non-transmissible diseases (NTD) kill more than 36 million people every year. Near 80% of the deaths by NTD are concentrated in the countries of low and middle incomes [20].

An increase in the incidence of non-transmissible chronic diseases exists. Cancer, obesity, diabetes, hypertension and cardiovascular disorders have been related to the consumption of food rich in fats, sugars and salt [19]. This is problematic in health and has led to the appearance of a series of food denominated healthful and multiple variants of them: foods of design (includes enriched foods), functional, nutraceuticals, pharmafoods, photochemical substances, chemopreventive agents, although the origin of this type of food can go back to very old times, of Hippocrates, when he postulated “Let your food be thy medicine, and let your medicine be thy food” [3,1]. The concept of “functional food” was developed in Japan in the mid-1980s. The terms “nutraceutical” and “dietary or food supplements” are not very popular in Japan as compared to most of other countries. However, the concept of “functional foods,” which benefit the structure and function of the human body, is known as a result of research studies initiated on the health benefits the structure and function of the human body andis known as a result of research studies initiated on the health benefits of food in 1984. The Ministry of Education organized a national research and development project to evaluate the functionalities of various foods. In 1991, the Ministry of Health and Welfare (MHW), now known as the Ministry of Health, Labor and Welfare (MHLW), introduced foods for specified health uses (FOSHU), for the control of such exaggerated and misleading claims [18]. FOSHU foods refer to a new categorization of some foods that proven through clinical and epidemiologists studies have demonstrated a positive effect in the prevention of some specific diseases [7].

Many expressions have been used to describe multiple natural products with effects on health. Some of these expressions are nutraceuticals, pharmafoods, designer foods, vitafoods, phytochemicals and foodcaeuticals, medical foods, dietary supplements and herbal

products/botanicals [16]. The U.S. National Academy of Science of the United States has defined functional foods like "any modified food or food ingredient which may provide a health benefit beyond the traditional nutrients it contains" [24]. Of similar form in the United Kingdom, the Ministry of Agriculture, Fisheries and Food has defined functional foods as: "A food that incorporates a component that gives it a certain beneficial medical or physiological property to him, different from its purely nutritious properties" [6].

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Mazza, G. [16] mentions that the "Health protection Branch of Health Canada,"the organism responsible for the protection of the health of the Canadian citizens, has suggested that the expressions functional food and nutraceutical product is used of independent from talk about these two presentation forms, according to the following definitions:

A functional food has an appearance similar to the one of a conventional food, is consumed as part of a normal diet, and in addition to its basic nutritious functions, it has demonstrated that it presents beneficial physiological properties and it reduces the risk of contracting chronic diseases.

A nutraceutical product is a product made from a food but sold in the form of pills, dusts, pharmaceutical potions and other presentations not associated generally with foods and that has demonstrated to have beneficial physiological properties or toprotect against chronic diseases.

The functional foods give a new approach to the nutrition and worldwide health, making it possible to improve the quality of life and decrease the public health problem costs [23].

"Functional Food" is one that has a specific function in the human organism by providing benefits; according to the functionality of some of the components of foods, these have been classified as: probiotic (microorganisms alive that promote benefits to the health), prebiotic (nondigestible ingredients thatbenefit the host), synbiotic (with "n" because it designates the mixture of prebiotic and probiotic with synergistic biological effects), nutrients (ingestion with preventive effect) and non-nutrients (bioactive compounds, most of them with antioxidant effect) [9].

CHARACTERISTICS OF SELENIUM

Selenium has been established as an essential trace element that is important in many biochemical and physiological processes [5].

Selenium (Se), in the form of selenocysteine, is the central structural component of a number of specific enzymes, making it an essential trace element for both humans and animals. A number of selenoproteins with known functions have been identified to date and include five glutathione peroxidases (GSH-Px), two deiodinases [11], several thioredoxin reductases and selenophosphate synthetase [2]. The importance of Se is mainly associated with its role as an essential part of the glutathione peroxidases (GSH-Px), which provide a defense against oxidative stress by catalyzing the reduction of organic hydroperoxides that react with the selenol group of selenocysteine [10]. In animals, and particularly in lambs, selenium deficiency has been linked to a number of diseases, which include white muscle disease and suppression of immunity [22]. The use of organic Se as a possible means of enhancing meat's Se content, particularly with selenoamino-acids, has not been largely studied. Concerning this specific subject, a new methodology including capillary HPLC and

inductively coupled plasma collision cell mass spectrometry to assess Se-Met and Se-Cyst in serum [8] and edible tissues [4] has recently been validated.

Research has shown that the organic and inorganic forms of Se vary in tissue retention and that organic Se is deposited in greater concentrations in many tissues compared with inorganic Se [12]. Selenium, as a part of glutathione peroxidase, protects against oxidative stress. The protection of cell membranes from oxidation may help in improving water-holding capacity of meat products. Mahanet al. [14] reported that 0.3 ppm of organic Se in basal diets with an endogenous Se content of 0.06 ppm reduced drip loss and improved muscle color compared with inorganic Se supplementation. However, there is still limited information regarding the effects of different Se sources on carcass characteristics. Furthermore, most of the recent studies [13,14] concerning the effectiveness of Se supplementation using different sources were conducted using feed ingredients with relatively low Se contents. Results have shown that even with the high concentrations of endogenous Se in the diet, supplementation of Se with organic source resulted in reduced drip loss from loins. The results of this study suggest that Se supplementation with organic Se may improve pork quality and provide greater Se intakes for consumers [15].

CONCLUSION

If using organic selenium in the feeding of the ovines, there is a greater availability of selenoproteins in the meat; it turns it into a food that provides proteins with antioxidants properties, which aidsprevention of the cellular damage in front of the free radicals, contributing in this way to diminishing the problems of public health when avoiding the presence of metabolites that contribute to the development of pathologies such as cancer, coronary diseases, or diabetes. It is necessary to do research that provides a deeper knowledge about the properties of the selenium like part of antioxidants enzymes that allow extending the shelf life of the meat.

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Chapter 11

ORGANIC CHROMIUM IN NUTRIENTS METABOLISM AND DISTRIBUTION IN SHEEP

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ABSTRACT

Chromium (Cr) is an essential trace element that can improve insulin action over basic cellular reactions to keep (homeostasis) in carbohydrates, lipids, proteins metabolism, and energy production. Previous research has shown that chromium organic compounds are biologically more important than inorganic salts; animal production has demonstrated improvements in growing, fertility, nutrient metabolism and immune response in different livestock species, but research in sheep are scarce; nevertheless thisresearch has shown improvement in growing and carcass quality. Thus is necessary for further research on possibly effects of chromium in other specific indicators of meat quality on sheep.

INTRODUCTION

Chromium (Cr) as a mineral element was discovered by Vaquelin in 1979; Vaquelin was a French chemist, who gave it such name because when it is treated with carbon at high temperatures, it shows a product highly colored [13]. Chromium is the number 21 considering its abundance on the earth cortex; it stands under several oxidation status, but the zero (Cr^{0}), trivalent (Cr^{+3}) and hexavalent (Cr^{+6}) are the most important ones in environmental and

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commercial products. Chromium zero is not present on the earth cortex, and it is biologically inert; chromium hexavalent is a hard oxidant agent, mainly in intermediate acidity; when this element reacts with genetic cell material, it is converted into a carcinogenic substance [24; 66]. Almost all Cr in nature is present as trivalent chromium (Cr^{+3}); in the majority of biological materials, it is associated tightly withproteins, nucleic acids, and other organism components [8]. There is evidence that the chromium, both in humans and animals, has a marked influence on carbohydrate, lipids and nucleic acids metabolism [51; 6; 78]; it can be part of an organometalic molecule known as glucose tolerant factor (GTF), whose structure contains chromium trivalent, nicotinic acid, glycine, glutamic acid and cysteine; the chromium has an active participation in the metabolism of carbohydrates, because GTF acts as a cofactor that increases the insulin action, facilitating its absorption and use in the cell [49; 58; 84]. Chromium is necessary in protein synthesis and corporal growth [52]; also, it improves productivity and energetic metabolism [35]. Several authors have demonstrated that supplementing with chromium improves nutrients' digestibility [42], increases muscular percentage, and decreases fat in growing pigs [66; 15; 48]. Other investigations have demonstrated that chromium increases fertility and live pig number in sows [45]. Studies in sheep, even though they are scarce, show high potential of chromium to improve carcass quality, decreasing both fat depots and cholesterol concentration in blood [39], bigger rib eye, and better productive response [27].

Organic chromium (as Cr^{+3}) seems that it is absorbed by passive diffusion in humans and animals at very low levels (0.4 to 3%) [17; 3; 4], but it shows an absorption coefficient of 10 to 25% as chromium chelates. The chelate can be defined as the complex formed between a binding and a metallic ion, where the binding (or chelant agent) should contain functional groups to be able to donate a pair of electrons in order for them to combine through a covalent union with a metal; among the most used organic chromium products (or chelated) are Cr-picolinate (Cr-Pic), Cr-niacin (Cr-Niac), Cr-yeast (Cr-yeast) and Cr-methionine (Cr-met)[43].

The greater effectiveness of organic chromium products over those inorganic ones becomes evident according to several investigations [10; 82]; in sheep, the investigation has focused on the effect of this mineral in nutrient metabolism (glucose, cholesterol, triglycerides), immunological status, and carcass characteristics [39; 31; 27]; however, there is not enough information about the effect of this mineral on meat quality in sheep; Cr effect on meat quality indicators has been evaluated only in species such as pigs and poultry, with increments in protein, water retention capacity, and lower fat meat content [10; 44; 38]; on the other hand, in ruminants it is not possible to establish an specific dose yet, but there have been benefic effects with Cr concentrations between 0.2 to 0.8 ppm [68; 37]. Therefore, it is evident that it is still necessary to investigate more in order to conclude about the effectiveness of Cr on the growing, carcass and meat quality in livestock species.

IMPORTANCE AND FUNCTION OF CHROMIUM IN NUTRIENTS METABOLISM AND DISTRIBUTION

The essential function of Cr in animal nutrition was recognized the first time by Schwartz and Mertz in 1959. These investigators in 1955, observed that laboratory mice fed with intravenous glucose developed intolerance to this carbohydrate; later, in 1957, Schwartz and

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Mertz informed that a substance, which they called *glucose intolerance factor* (GIF), present in *Saccharomyces cereviceae* (beer yeast) could eliminate this problem. They identified the Cr as an active component of GIF; in that year, they isolated the GIF from a yeast hydrolysed as well as from the kidney of the pig; then, when this component was introduced to the mice stomach, which had glucose intolerance, through a catheter, they observed the metabolism of this aldohexose was re-established. Further, in other studies, it was informed that the chromium in the GIF yeast was absorbed more easily than in the sources with inorganic chromium, and it was proposed that the GIF yeast was composed of chromium ions, nicotinic acid, glycine, glutamic acid and cysteine. Additionally, it has been proposed that the GIF yeast in an isolated form can be the result of the acid hydrolysis in special proteins, which bound the Cr [52; 81]. The GIF action mechanism is not very clear yet, however, it is likely that this acts by facilitating the linking of the insulin to the cellular receptors, and when this happens, the glucose insertion to the interior of the cells is facilitated (hepatic, muscular and adipose tissue) so that it starts with its metabolism (anabolic or catabolic); that is why this organometallic component (GIF) contributes to the obtaining of energy starting from this carbohydrate, in addition to contributing to the regulation of the glucose levels in the blood [53].

The insulin that is secreted by the beta cells in Langerhans clusters of pancreas has a very important anabolic effect (liver, muscle and adipose tissue), besides inhibiting the gluconeogenesis. In the first case (anabolism), the glucose, once inside the cell, must follow different metabolic routes so as to become in glycogen, aminoacids (non-essential) or fatty acids; in the second case (gluconeogenesis), it impedes obtaining glucose from proteins and glycerol [80; 19; 51].

Great progress has been recently achieved to understand the molecular base of the action of Cr in the regulation of the metabolism in carbohydrates and lipids. A peptide known as a substance that binds low molecular weight chromium (LMWCr) was isolated, and it is believed that it plays a very critical role in the modulation of the insulin action on its receptors; this peptide seems to be widely distributed in tissues in mammals and has been isolated from different sources [84]. Yamamoto et al.[85] isolated and characterized this oligopeptide, which binds chromium; it was denominated as the substance that binds low molecular weight chromium (LMWCr) or chromoduline as it is known nowadays [84]. This oligopeptide has an approximate molecular weight of1500Da, and it is made up by four kinds of aminoacids (glycine, cysteine, glutamate, and aspartate), and it binds four kinds of chromium. The release of chromoduline is similar to the hormonal secretion since it is released in the blood stream as a response to a stimulus that can be the hyperglycemia [83]. Chromium ions transportation is carried out through the transferrin protein as a response to the increase in the plasmatic insulin concentrations [84]. The main action of chromium, as a chromium-chromoduline complex, is through a system of auto amplification of the insulin signal due to the activation of the tirosinace region in the subunit beta of the insulin receptor, which modifies the glucose capture and the lipids metabolism [25]. The action mechanism proposed by Vincent [84] for the activation of the tirosinace region in the insulin receptor starts with the binding of the insulin to its receptors, which allows the entry of the chromium in the blood to the insulin dependent cells; inside the cell, the chromium binds to the apochromoduline, and it is transformed into chromoduline, which in turn, binds to the insulin receptor and activates the cascades of the kinases. Once the concentration of insulin decreases, the chromoduline is released from the receptor and reverses its effects (Figure 1).

There is no evidence that the chromium is a component of the subunits of the receptor, nor that it acts as an accessory for the binding of the insulin with its receptor and neither that it acts as a second messenger in the mediation of the effect of the insulin [25].

CARBOHYDRATES METABOLISM

The supplementation with Cr and insulin to animal tissues within *in vitro* experiments has led to the increase of glucose oxidation, providing as a result CO_2+H_2O, the increase in the glycogenesis as well as the glucose conversion into lipids, all this together with the increase in the glucose utilization[8].

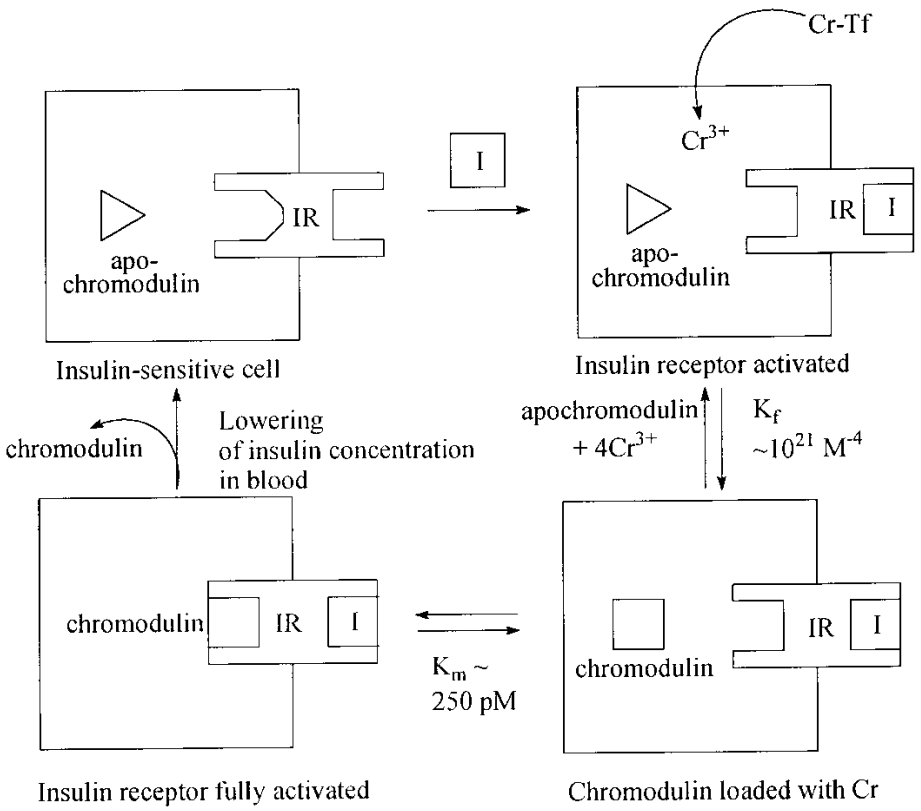


Figure 1. Proposed mechanism of action of chromium and LMWCr (substance that binds low molecular weight chromium) promoted by insulin action [84].

LIPIDS METABOLISM

There is evidence that Cr is essential to the lipids metabolism as well as to reducing the risk of atherogenesis. For example, in the case of rats and rabbits thathave been fed with a low Cr diet, it showed an increase in their total cholesterol as well as the concentrations of lipids in the aorta [1]. On the other hand, McNamara and Valdez [47] studied how the Cr

propionate worked in the lipogenesis and lipolysis in adipose tissues in Holstein cows, starting with 21 days pre-parturition and lasting until 35 days post-partum. The Cr increased the net synthesis of fat in adipose tissues, and it also diminished its net release. This can be due to the actuation, through the chromoduline connection with the insulin receptor and the increase in the glucose flow in the interior of the adiposities.

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PROTEIN METABOLISM

Evans and Bowman [29] demonstrated an increase of glucose and amino acids absorption in musculoskeletal of rats that were fed with Cr-picolinate. This alteration in nutrient absorption was associated with insulin parameters alterations that are chromium dependent. These observations can explain the glucose tolerance effect and the increment in percentage of musculoskeletal reported by some researchers. The improving absorption potential of amino acids by muscular cells benefits the total protein deposition. Roginski and Mertz [72] said that chromium supplementation intensifies the aminoacids incorporation into hearth proteins and in rat tissues.

NUCLEIC ACIDS METABOLISM

The trivalent chromium seems to be involved in the structure and expression of genetic information in animals. Chromium connection with nucleic acids is more strength than other metallic ions [62]. Chromium protects the RNA from heat denaturalization; furthermore, it concentrates in cellular nucleus. Chromium has increased *in vitro* RNA synthesis in mice [63], which supports the hypothesis that it has effect on the genes function. It participates in the gene expression through the linking with chromatin and, as a consequence, with an increment in RNA synthesis, because it induces nucleus linked protein and nuclear chromatin activation [69].

MINERAL SUBSTANCES METABOLISM

There are few reports on Cr supplementation effects in metabolism of mineral substances. The Cr and Fe relation has been investigated since it became evident that both minerals were transported by the transferrine protein [84]. At low Fe saturation, Cr and Fe tighten each one to different union sites. However, when Fe concentration is high, both minerals compete for the same linking sites. The evidence that Cr can influence on Fe metabolism has been described by Ani and Mostaghie [9]. The Fe homeostasis alteration has been reported by other authors; the most significant was detected in Cr-picolinate supplement association [46]. The Fe metabolism alteration in association with Cr supplementation was reported by Anderson [7], who identified a decrease in Fe concentration in tissues as a response to Cr supplementation.

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BIOAVAILABILITY OF CHROMIUM

Chromium can be absorbed through digestive, respiratory and skin way. The intestinal (jejune) Cr absorption is low, both in humans and animals, and varies from 0.5 to 2%, depending on daily dietary intake; the rest is excreted in feces, urine and bile; the normal excretion through urine is 0.05-0.5 μg/day. The mechanism of absorption in not known with exactitude, it is believed that it is by diffusion or through transporter proteins. Once chromium has been absorbed, it is distributed and stored in several tissues, with greater concentrations in kidneys, muscle and liver; it is also found in spleen and bone tissue [20]. Aminoacids like methionine and histidine, as well as vitamin C, enhance Cr absorption, but phytates inhibit it [28]. High carbohydrate ingestion, gestation, lactation, different infections, diets with low protein concentration, stressful factors like heath, physical trauma and extreme exercise (transport) are considered like agents that alter chromium metabolism, increasing its excretion in urine [16; 5]. The losses of chromium in urine can be used as a measure of the response to the stress in the organism, since the Cr is mobilized as a response to a particular stress and not reabsorbed by the kidneys, and it is lost in the urine [2].

CHROMIUM USE IN ANIMAL PRODUCTION

In the beginnings of the 90s, a series of studies started with zootechny objectives in domestic animals; the interest of these studies was targeted towards taking advantage of the Cr salts lipolitic effects. The positive results obtained in pigs [65] promoted its utilization in cattle; in the case of this species, the most outstanding results were associated withthe effects in the health and immune function; during the stress periods, not only were the levels of cortisol reduced, but also the immuglobulines production was increased [21; 55].

EFFECT OF THE ORGANIC CHROMIUM IN THE PRODUCTIVE RESPONSE

The interest to determine whether the supplementation with Cr in organic forms does improve some of the productive aspects in cattle, sheep and pigs is based on its function as a glucose tolerance factor, in such ways that it favors the insulin action, with faster glucose absorption [67]. Even though the meat price is usually established by the marked based on carcass kg, commercial transactions have a tendency to be performed, so much, on the living weight as on the carcass weight of the animals, which initially is the one to set the economy value of an animal, and therefore, the benefit for the producer; it is not the whole deal, the conformation and other carcass characteristics can also modify the value of the animal [32]. The Cr in pigs has had a better response than sheep and cattle; however, there is a lack of investigation to produce definite conclusions about the effectiveness inimproving the animal performance, as well as the immune state and the carcass quality [51]. Kitchalong (39) did not find any differences in the productive response when adding 0.250 ppm of Cr to the Suffolk sheep diet; in the same way, Gentry[31] supplemented Suffolk sheep with PicCr and different protein levels and reported no effects on the productive response either; on the other hand,

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Arvizu [12], evaluated the effect in proteic and energetic supplement in grazing sheep Suffolk x Dorper and Rambouillet, as well as an organic Cr source, indicating that none of the variables in the growth were affected by the Cr; in a similar study, Moreno [56], when evaluating different levels of an organic Cr source (yeast rich in Cr) in the Sufflok sheep diet, did not indicate effects in any of the variables in the productive response either. However, there are some investigations that have proved the effectiveness of the Cr supplementation on some growth aspects; Juarez [37] obtained an increase in the daily weight gain in hair sheep with a Cr methionine supplement, in a dose of 0.35 ppm; Chavez and Garcia [23] evaluated the effect of Cr organic sources (Bio-chrome) and selenium (Sel-Plex-50) on the productive performance as well as *in vivo* dry matter digestibility in intensive feeding growing sheep, and they found that the daily weight gain, feed conversion, final weight gain and total weight gain was improved; Dominguez [27] indicated a linear decrease in the dry matter intake (DMI) and in the feed conversion, where as a linear decrease was observed in daily gain and final live weight, due to the effect of the organic Cr in¾ Rambouillet sheep, having an increase in the effect of the Cr when yeast rich in Se was used.

There is a series of factors that may have caused these discrepancies when using Cr in the diet, as well as in the length of the period of supplementation, the nutrimental composition of such diet [31], the chemical structure or the Cr source, which may vary its bioavailability [65]; besides, the most consistent benefits of Cr in the productive response have been associated withanimals under stress; there is some evidence that the Cr in calves diet under stress because of the transportation and vaccination has improved the initial weight gain and the immune humoral response [55]. On the other hand, the Cr essentiality was proven for the first time by Schwarz and Mertz [77] in rats, and the importance in humans was demonstrated by Jeejebhoy [36]; however, the requirements in the diets of livestock species has not yet been established. The importance of the Cr supplementation levels is made evident in a study in which the addition of 0.2 mg/kg DM slightly improved the performance and carcass composition, but when a dose of 0.4 mg/kg DM was used, no effects were found [54]; Pollard andRichardson [68] indicated that 0.2 ppm of Cr (yeast rich in Cr) improved the feed conversion (FC) of fattening cattle, but no positive effects were found when the dose was doubled to 0.4 ppm of Cr; Juarez [37] studied Pelibuey sheep supplemented with Cr (Cr L-methionine) and reported that the daily weight gain was increased when the dose was modified from 0.4 to 0.8 ppm. In contrast, there are some studies [39; 27; 74] where the inclusion of Cr in a dose of 0.25 ppm (CrPic), 0.2 ppm (Cr-L-methionine) and 0.25 ppm (yeast rich in Cr) did not improve the productive response in finishing sheep. Such differences may be attributed to the chemical structure of the Cr, and they also suggest that it is still not possible to establish an optimal dose of Cr for feeding ruminants. On the other hand, the available information on the Cr levels in animals is not easy to measure; the Cr concentration in the blood has not been evaluated either, and the ingested Cr in the diet, the absorbed one and the one that has been metabolized, can be affected by a great number of factors; therefore, the content of ingested Cr is not proportional to the quantity of available Cr to enhance the insulin action [18; 57; 34].

EFFECT OF THE ORGANIC CHROMIUM IN THE CHARACTERISTICS OF THE CARCASS QUALITY

The effects of the supplementation with Cr on the growth and quantity of lean carcass have motivated still more interest than the effect of the Cr on the reproductive parameters [67]. The carcass is only an intermediate step in meat production as a final product. Many pieces quality will depend on the quality of the carcass, and this quality associates the carcass criteria withthe meat quality [32]. The value of the sheep carcass depends on many factors, specially the weight, conformation, proportion of the main tissues (muscle, fat, and bone), distribution of the latter ones in the carcass, the muscle thickness and the meat quality. The evaluation of the carcass allows improving the meat commercialization since it makes an efficient relation between a good product and a demanding consumer [40]. There area great deal of investigations where the positive effects of using different sources of Cr in diets for livestock species such as pigs and ruminants are explained; Kitchalong[39] indicated that when adding Cr tripicolinate to a dose of 0.25 ppm to Suffolk lambs, the fat level in the tenth rib was reduced by 18% (7.2 to 5.9 mm); Dominguez [27], by using Cr and Se organic sources (yeast rich in Cr and Se), mentioned that the rib eye in ¾ Rambouillet sheep, which received Cr, was from 1 to 3 cm^2 bigger in sheep that had not been received Cr and that the dorsal fat was reduced from 2.6 to 1.4 mm by the effect of Cr; Arvizu [12], when evaluatingthe effect of a proteic and energetic supplement in Suffolk x Dorper and Rambouillet grazing sheep and an organic Cr source, reported a reduction in the dorsal fat by an 18.4% due to the effect of the Cr; Gardner [30] indicated that adding organic Cr in diets for sheep decreased the subcutaneous fat in a level of the 12^{th} rib by a 20%, but there was no effect in any other carcass variable; on the other hand, Moreno [56] did not observe any difference due to the effect of organic Cr levels in the rib eye such as fattening or carcass muscular conformation. However, the perirenal fat was different between treatments; at the same time, a linear increase in the perimeter of the leg due to the Cr was observed. Dominguez [27] reported that 0.2 ppm of Cr supplied as Cr L- methionine did not increase the rib eye and did not decrease the dorsal fat in finishing sheep carcass. It is important to remember the influence of Cr in the metabolic processes as well as in the incorporation of aminoacids to proteins as to increase the efficiency of the protein use by the cell in the organism, increasing the protein retention and deposit in tissues, which in turn comes to the muscular mass [50]. On the other hand, the differences regarding the improvement in the deposition of the lean tissue and the decrease in the gathering of fat in some studies has been related to the way in which the Cr is supplemented; dealing with this topic, Page [65] indicated that in pigs, the addition of CrPic increased the area of the long dorsal muscle and the percentage of the muscle and also reduced the fat level of the tenthrib; while the addition of Cr chloride did not show any effect whatsoever; Mooney and Cromwell [54] observed that the Cr picolinate was more effective than the Cr chloride in order to improve the characteristics of the pigs carcass. On the other hand, it is important to consider that the intramuscular fat thatis present in the meat allows distinguishing some ideal sensorial characteristics, such as the juiciness, tenderness, flavor and digestibility; the intramuscular and intermuscular adipose tissue contributes to the meat firmness, and a greater intramuscular fat content provides more tenderness and juiciness in the meat [60; 75].

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The carcass weight is a characteristic that creates great interest since it has some influence in its conformation, fattening, tissue composition and pieces proportion and therefore affects directly its quality and price. It is directly correlated with the slaughter weight, and this must coincide with the point of maturity in which a particular breed reaches the optimum or ideal level [14]. The carcass weight is the one that determines the commercial value, since the industry commercializes on the basis of kilogram price [33]. Kitchalong [39] and Dominguez [27] did not report effects due to the levels of Cr on the sheep diet in variables such as the slaughter weight or cold and hot carcass weight and on performance of the carcass; however, Pollard [69] indicated that 0.20 ppm of organic Cr in feeding calves, increased the hot carcass weight, while 0.40 ppm of Cr produced carcass with less fat and less marbling.

EFFECTS OF THE ORGANIC CHROMIUM IN THE MEAT QUALITY CHARACTERISTICS

Meat chemical composition has a special relevance in the quality of this food product, since it has a wide range of nutrients (proteins, fats, water, minerals and vitamins); on the other hand, the chemical composition of the meat is important since it affects its technological, hygienic, sanitary and sensorial quality [76].

As it was already indicated, there is scarce information on the result of the Cr in the meat quality of sheep, even though Cr has demonstrated positive effects on some meat quality indicators in species such as pigs and poultry. Amatya [10] mentioned that by supplementing a poultry diet with different Cr sources (potassium chromate, Cr chloride, yeast rich in Cr) at a dose of 0.2 mg/kg DM, the concentration of protein in the meat proved to be bigger in groups thathad been supplemented with Cr compared with the control group; besides, the fat decreased in the groups supplemented with Cr. In the same way, Lien [44] and Kim [38] pointed out that by supplementing with 1,600 ppb and 200 to 400 ppb of Cr in poultry, there was a reduction in the protein concentration in the poultry meat when they had been fed with Cr being under stress conditions. On the other hand, Arvizu [12] indicated that when evaluating the effect of a proteic and energetic supplement and an organic Cr source in Suffolk x Dorper and Rambouillet grazing sheep, the Cr reduced by 15.3% the fat found in the meat; in contrast, Moreno [56] did not report any effect on the level or organic Cr in Suffolk sheep in the content of humidity, protein or fat in the meat;however, the level of ashes in groups supplemented with Cr was smaller compared to the control group, which may indicate a reduction of the total mineral content in the meat with Cr; nevertheless, this does not offer a real idea of the mineral content since the determination of ashes provides only an estimate of the total content of such elements.

In general terms, the meat quality includes those sensorial characteristics that make the meat a tasty product for humans, such asthe tenderness and juiciness [61]. On the other hand, both the final value of pH (approximately after 24 hours of slaughtering) as well as the decreasing speed of it during the transformation of the muscle into meat affect the organoleptic characteristics (color, juiciness, flavor) and technological characteristics (capacity of water retention); the determination of this parameters constitutes a good measure to know the maturing process and to value the meat quality as the final product [70]. This is

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how a product with an elevated final pH produces meat that presents a dry external consistency of muscle, hard and sticky; in these conditions the useful shelf life period of meat cuts is reduced, since it is associated witha high water retention capacity, which favors the bacteria growth [11]. Toghyani [82], when comparing two Cr sources (Cr nicotinate and Cr chloride) in different levels, indicated that the meat pH was not influenced meaningfully by the source or dose of Cr; nevertheless, it was possible to observe a tendency to increase the pH in the Cr supplemented groups, especially the group with Cr chloride; in the same way, Moreno [56] indicated that the pH did not have a meaningful variance due to the effect of the Cr; however, a linear increase in the initial pH due to the use of Cr was observed. Amatya[10] used different Cr sources (potassium chromate, Cr chloride and yeast rich in Cr) in poultry, and they did not find variation in meat pH as a cause of the Cr sources. The water retention capacity influences the meat quality and it is related to the texture, the tenderness and the color of raw meat, as well as the juiciness and firmness of the cooked meat [59]. Ria [71] did not find any effects of Cr on the water retention capacity when adding Cr propionate in different phases of the growing pigs and poultry; in contrast, Amatya [10] indicated an increase in the water retention capacity in poultry supplemented with 0.2 mg/kg DM of Cr (yeast rich in Cr).

The consumer considers that tenderness is the most important characteristic in the meat quality, which is supported by the fact that they are willing to pay more for more tender cuts [41]. Ria [71] indicated that by adding Cr propionate in different growing phases in pigs, the Cr decreased the sheer force; nevertheless, the author indicated the result influenced the addition of a fat source in the diet; in contrast, Arvizu [12], when evaluating in Suffolk x Dorper and Rambouillet grazing sheep, the effect of a proteic and energetic supplement and a Cr source, indicated that the sheer force was not affected by the Cr concentration;the same result was reported by Moreno [56] in Suffolk sheep, when the sheer force was not affected either by different levels of an organic Cr source. Amatya [10] supplemented the poultry diet with different Cr sources (potassium chromate, Cr chloride, yeast rich in Cr) at a dose of 0.2 mg/kg DM and evaluated different sensorial attributes of the meat; then, they reported an improvement in the meat sensorial evaluation, animals that had been supplemented with Cr; meaningful effects on the evaluated parameters in the yeast rich in Cr supplemented groups are also mentioned; on the other hand, seen from a practical point of view, it is necessary to carry out a complete sensorial analysis in the meat of sheep treated with Cr to determine if the consumers can distinguish a numerical difference expressed by the sheer force.

CONCLUSION

There is enough evidence that the Cr is an essential element involved in the normal functioning of the carbohydrates, lipids and proteins metabolism. However, the results in the use of Cr in its biologically active forms regarding the livestock species are still not conclusive. The positive effects associated with the use of Cr include growing improvements, increments in the muscle proportion and lowering of carcass fat, as well as some improvements in certain parameters regarding the meat quality. These benefic effects have been evaluated in different livestock species, in different growing phases and levels of stress, using different biologically active levels and sources of Cr. On the other hand, the results in

which no benefic effect has been observed due to the Cr supplementation are useful to point out that the available information about Cr content in feed ingredients for animals is scarce and variable; also, the status and concentrations of Cr in blood in livestock species have not been evaluated enough, nor has been the interaction of Cr with other dietetic factors. It is obvious that these points need to be investigated still to produce definite conclusion on the effectiveness of this mineral in the different livestock species, including sheep.

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Chapter 12

PROBIOTICS AS AN ALTERNATIVE TO ANTIBIOTICS PROMOTERS IN RUMINANTS

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ABSTRACT

Dairy production is considered to be a complex activity integrated into a production system, geared to meet the demand for milk and dairy products dairy in animal and human food. It is characterized by a sustainable and organized process that allows keeping the productive efficiency of the dairy company. Raising calves of replacement, ensures the profitability of the company dairy and beef companies. The period of lactation of the calf is a critical stage in the process of adaptation of the newborn; there is a need to transfer infant transcalostral of immunoglobulins, which ensures passive immunity and contribution suitable nutrient in the cow's milk or from a diet feeding in the lactosubstitutos. Coupled with this are important structural and functional changes in the stomach above the calf;, they allow the evolution of the digestive system of the nursing monogastrico to the ruminant developing. This change is accentuated by the colonization of the microflora and fauna of the stomach, the abomasum and bowel. Induction of the microbiota in the gut is produced from : from the production environment and, the coexistence between animals and microbial additives included in the diet. The biomodulactióon of the microflora of the digestive tract of calves, by the use of additives such as probiotics, has beneficial effects to the host. Through the bioregulaciòn bioregulation of the microflora and the inmunomodulaciòntion of the adaptive immune response and interaction with innate immunity of the individual—: which happen to

adverse health conditions—; attributed to enteric diseases and intestinal dysbiosis, among others. The use of probiotics, prebiotics and symbiotic in animal feeding is to promote a beneficial microflora in intestine and rumen. An Oother effect is to contribute to the bioregulaciòntion and inmunomodulaciòntion of the immune response in the intestine by the colonization and transit of microorganisms in gastrointestinal contents. Another considered effect is to promote the microbiota and their lLietabolic processes, as a beneficiusin digestive process in the prerumiante and the ruminant adult. Ruminal microflora, are required for the synthesis of nutrients and the development and the fermentation desired, in the sustainable production of livestock. The use of probiotics as additives adaptogenos is the process of adaptation of infants to the production system, in addition to constituting an alternative appropriate substitution of the antibiotic promoters of growth in the rearing of calves.

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INTRODUCTION

World milk production is considered to be a complex and dynamic activity, geared to meet the demand for milk and products dairy in the human population. Maintaining cattle system to sustainable production, allowing maintains for the maintenance of the productive efficiency of the dairy company and the feedlot production. To maintain the productive livestock inventory, it is necessary to ensuring ensure its animal welfare and health of the dairy herd [158]. To preserve the production environment and the quality and food safety of products of animal origin [37, 159, 163]. T, the current challenge that has with dairy and meat, is to keep the keep production responsible and sustainable; and to maintain the productivity and competitiveness of the cattle farms, in the global market of agricultural foodstuffs [18, 54, 77, 125]. Considering the importance of milk and dairy in human nutrition [46, 68, 131]—, which generates a growing demand for products of quality healthy and safety food for consumption and is, influenced by the new preferences of consumers and changes in consumption patterns, modified by cultural, socio-economic and geographic factors in the population human [131, 145, 147, 167]—r. Replacement of the stock of production animals farming is of strategic importance, which allowsallowing for efficient production to ensure profitability for company dairy and meat companies. The period of lactation of calves is considered critical by to the adaptation process of the neonate. The adaptive process of the newborn, is set based on the need to provide the conditions of ambient comfort and that is suitable for its animal welfare [7, 117]. In addition, it is necessary that there is to an adequate supply of colostrum of quality for the passive transfer of maternal immunity through the present in calostrum immunoglobulin, which are is essential for the survival and the state of health of the newborn calf [64, 154]. Likewise, the morphofunctional changes of the stomach above the calf during breastfeeding. They are fundamental to its functional transition of a monogastrico animal at birth, to a ruminant young at weaning [2, 49].

Breastfeeding digestive and respiratory diseases occur more frequently, leading to an increase in production costs, as well as affecting the quality of the rearing of calves of for replacement. The use of probiotics and prebiotics in the diet during the lactation, favors the adaptive process of lactating animals loa, improves the microflora, and the digestive process and stimulation ofes the immune mechanisms. The mixtures of probiotics and proebiotics, as well as certain strains of probiotics to improve the Sstate and the general condition of the animals in breastfeeding, for this reason the use of probiotics is a sustainable alternative

which that can replace the use of antibiotics as growth promoters in calves [7, 35, 36, 48, 110].

MORPHOLOGIC AND FUNCTIONAL CHANGES OF THE DIGESTIVE TRACT

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In the first stage of his life of nursing calves in the adaptive process adapting to the environment of production, the digestive tract undergoes structural and physiological changes that ensure the transformation of the digestion process monogastric, to the of a ruminant shortly after birth [136]. Thereo occur a greater anatomic changes during the development of the previous digestive, in particular the proportion omaso/rumen [133]; which during the first week, the proportion abomasums to rumen is to 5: 1, for the four weeks from 1: 1 and after twelve weeks, this proportion between the omasum and the rumen is reversed to 1: 5, compared to existing during the first week wing, there are also important changes in morphofunctional, who suffers the anterior of the pre stomachs [87, 133]; there are other observed changes in the ruminal mucosa epithelium amending, by functional esophageal leak closure, rumino-reticular function increasing and the growth of the papillae ruminal for the fermentation and absorption of volatile fatty acids (VFA), influenced by the main source of energy of ruminants throughougth rumen fermentation of forages and grains [38].

The abomasum is the principal organ of the gastric digestion in the pre-ruminant phase in the calves during lactation milk or I milk replacementsrs; it is the, single supply of nutrients in the diet during their growth. This phase extends from birth to 3 three weeks of life, at the start of the calf eating solid foods;, this period may be extended if it backtracks the consumption of solid foods. On the functional development of the stomach above, the calf can demonstrate by papillary rumen development, accentuated by metabolism of the acid butyric, and by the papilla ruminal epithelium. During this period, three phases can be distinguished [168, 171].

The first phase of gastric, characteristic of the consumption of milk digestion or her formula milk des of birth, the only solid food consumption is in the form of concentrated approximately three weeks of life. The second transitional phase of prerumiante of is the start of the consumption of concentrates and access ad libitum of water, along with the milk feeding.; During this phase starts fermentation rumen and rumen development. The third stage of ruminant isn full sets from weaning and is perpetual until the end of his life, characterized by fodder consumption and concentrates concentrating onfor the support of the ruminal digestive process, producing large amounts of VFA and microbial protein through the rumen fermentation [59]. While other carbohydrates and other nutrients such as protein overweight go through the abomasum, wing lower from the intestine to its degradation by pancreatic juice and other intestinal enzymes [26, 38, 39].].

Rumen develops from the non-secretory portion of the abomasum, with a motility perfectly differentiated of the abomasum, and consequently the implementation of the ruminal biomass in symbiosis with the host [133]. Various studies show that calves are ready for weaning, close to the two months when there is a greater aptitude morfofisiológica of the rumen and the absorption capacity of the intestinal tube is better. This functional ability can be induced primarily by the quality of the diet and microflora that colonizes in digestive tract

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of the newborn. Also, the physiological and, chemical changes are ocurrigoccurring in the digestive tract influenced by mechanical stimulation of the rumen [136]. Some authors consider that it is feasible to make weaning when the calf weighs between 45 and 50 kg of live weight, an intake of 1.5 to 2 of the live weight, as well as a minimum sustained consumption of 900 g daily of solid food of excellent quality [117]. In the newborn calf, rumen microbial is very poor, its implantation occurs during the first weeks, depending in large part by the regime of the diet. In the early days of born life, the imperfect closing of esophageal leak, small amounts of milk fall for reticular canal to the rumen, serving as substrate for the colonization of microorganisms. To subsequently increase the consumption of food and water creates conditions optimal in the rumen for incipient ruminal fermentation in calves and production gradually VFA, accompanied by microbial activity ruminal at a pH of 6.4 to 7.2 t the optimo papillary development of the rumen, influenced by consumption of concentrates and high quality forage [38].

The esophageal leak is a muscular projection that communicates to the esophagus and the abomasum along the lattice without going through the previous diverticula of the forestomach earlier in development. The closure of the esophageal leak, caused by vagal stimulation, by breastfeeding, liquids from the diet are driven by the canal of the esophagus to the omasal sac and finally to the abomasums [87]. Calves drink water, the esophageal leak, is not stimulated and water passes primary way wing preestomago and their compartments. When administered with antibiotics and antiparasitics, these can be provided with the Ovo replacement or administered with some compounds in solution such as copper and zinc sulfate and sodium bicarbonate, to induce the closure of the esophageal leak [133].

The closure of the esophageal leak in the nursing calf, diverts the flow of milk ingested into the interior of the abomasum;, this change of route allows milk to reach the abomasum without chemical Treasury treasury modifications and integrates in its nutritional properties [3]. To get the milk to the abomasum, it coagulates quickly, before the 10 ten minutes by the action of Renin, depending on the quality of the milk substitute. When the proportion of milk is high in the ovo substitute, there is a greater amount of Renin in the abomasum, compared with those containing a proportion of whey may. However says that the formation of the clot is not essential, when the quality of the protein of the lacto replacement is very good. Although the digestion of milk protein in young calves, is basically due to the action of Renin, pepsin and hydrochloric acid [7, 39].

At the time of the clot of milk in the abomasum, casein arises and passes into the duodenum, along with casein. The clot formation occurs at the level of the abomasum to occur the reaction between casein and calcium dairy product forming the paracaseinato of calcium, which captures the fat in milk. Milk clot is favored by close to 6.0 abomasal pH and a proper abomasal motility, which contributes to the release and the passage of the serum into the small intestine. The pH of the abomasum in the nursing calf fasting is found in a range of pH of 2.0 to 3.0 units, one hour after eating the pH is increased from 4.3 to 6.0 [3, 38, 147].

It has been suggested that the fault in the production of rennet in the digestion of milk predisposes to infection by pathogenic strains of *E. coli* for dyspepsia and abomasum achlorhydria. The optimum pH for coagulant action of milk by the gastric enzymes in milk is 6.5 to Renin and 5.2 for pepsin. Proteolytic degradation of the clot of milk is optimal at pH 3.5 to Renin and only 2. 2 to pepsin [39, 136].

The establishment of the calf rumen function prior to weaning, biomass is composed of protozoa and bacteria that accentuate the activity aminolitica and celulolitica of the rumen in

a range of pH of 6.4 to 7.3, which is favourable for microbial activity [147]. Reticular rumino movements are asked to establish the rumination and increase the thickness of the wall; the tone of the rumen is perceived derived from ruminal digestion by the consumption of solid foods and water, as well as an appropriate proportion of quality forage [38].

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RUMEN FERMENTATION IN THE CALF

There is a constant supply of food and water, which creates an enabling environment for the fermentation of rumen to an optimum temperature of 39 °C and pH rumen without much variation; between 6.9 and 7.4 and an appropriate osmolarity, remains a redox potential because of the intense microbial activity and the low voltage of oxygen in the gas phase, microflora is anaerobic, they play essential in the metabolism of rumen in cellulosic digestion and starches, important for the production of VFA in the absence of chitinases in the digestive tract of ruminants [28, 29, 136, 147].

The use of forage and concentrates on feeding calves favours the early development of the rumen function, the incorporation of the microbiota digestion ruminal for the use of fodder ligno cellulose, and starch and soluble carbohydrates for the production of VFA [164]. Similarly, the biomass of the ruminal microbiota joins wing microbial, a useful protein source to keep the animal protein balance. In addition to the synthesis of cofactors such as the cobalamin and thiamine, among others [16, 17]. To increase the intake of dry matter, decreases in urinary excretion of water and fecal excretion of water increases the major step content rumen and increased secretion of water through the gut. Associated with an increase in water intake, it is possible that the increase in osmoreguladoras substances in the blood, such as glucose, the level of VFA and certain electrolytes and minerals, which stimulate the secretion of antidiuretic hormone [24, 31].

In this regard of the early inclusion of forage in feeding the calf, some researchers conclude that it is not essential for inclusion of fibrous material in calves before weaning, considering that if the feed is of poor quality it can be a problem to displace functional rumen biomass. However in many studies, they support management of fodder of good quality as alfalfa hay stimulates the consumption of dry matter, develops the microflora and fauna, to increase fermentation ruminal, VFA production and digestive ability of rumen [11, 15, 36].

MICROFLORA IN NEWBORN CALF

The intestinal microflora, acquired in the neonatal period of dairy cakfcalf, remained stable during his lifetime; some factors such as changes in diet and the use of antibiotics could have negative effects on your balance. The addition of bacteria in the form of probiotics tends to positively alter the flora and the proportion of beneficial bacteria in the digestive tract improves the balance in the intestinal microbiota and rumen [28, 29, 39].

The biomass of the microflora rumen and intestinal cattle is acquired by the environment and of life contact with other animals during the period of lactation, where settlement occurs and primarily anaerobic sporulated, *Clostridium ssp* and *E. coli*. In some cases, colonization by pathogens predisposes to the development of clinical consideration enteric infections [39,

169]. One of the main benefits of the microflora effects is the synthesis of B-complex vitamins; thiamine, riboflavin, Pyridoxine, Pantothenic acid, folic acid and cyanocobalamin among others, as well as the synthesis of vitamin K. Bacteria such as *E. coli*,, and *Lactobacillus* ssp, *Bacteroides* ssp apatogenas., can help regulate the microflora through a process of competitive exclusion, production of organic acids and pathogens. I, in addition to stimulating innate immunity in the intestine with the secretion of mucus and proteins of the type of the defensins [136].

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In monogastric, like nursing calves, the microflora of the colon is composed by of a high proportion of *Lactobacillus ssp*. and Bifidobacteria, which synthesize a large proportion of fatty acids in short chain, product of the lactic acid fermentation of carbohydrates present in the colon and the blind, to avoid the residence of pathogenic bacteria [160].

The influence of dry food consumption and the abomasum content of the young calf has a varied population of *Lactobacillus*, product of regurgitation of the abomasum to rumen milk, w. Which promotes the initial introduction of the ruminal microbiota; *Lactobacillus ssp*., *E. coli* and *Cl. welchii*. Other aminoliticas such as *Streptococcus* ruminal bacteria ssp., are not set if not until that the calf consumes larger amount of solid food [31]. Some oOne of the sheep feed additives, like a plant extracts obtained from Acacia saligna, to produce microbial changes of the lactic acid bacteria AL-B; *Lactobacillus plantarum* y *Enterococcus faecium*, were explained by the contet of tannins of the plant extract and the metabolic adaptability of the biomass, with the changes in diet and nutrient availability in the media [142].

Microbiota in the newborn calf rumen, is low in the first weeks of life, the increase of biomass depends on several factors such as: diet, the fall of milk to the rumen by reticular canal during feeding and continuous reflux of the abomasum rumen content, creatinge conditions conducive to the development of the microflora and micro fauna of rumen in next to weaning calves [38].

When the provision of solid food and fodder is constant, as well as the free consumption of water, there is a steady supply of food and water that creates an enabling environment for bacterial symbiosis and ruminal fermentation. The inclusion of solid foods in the diet, in particular concentrated calves, during the first two weeks increases the rumino-reticular functional development of calves fed with concentrated, in comparison with the lambs fed with high levels of milk, between 12 and 15 of the its body weight [3, 59]. Similarly, the addition of grains in the diet increases the rate of growth related to the increased production of VFA. These conditions of management during the rearing of calves favor early weaning to produce an early adaptation to the consumption of concentrates [38].

DIGESTION OF CARBOHYDRATES IN CALF

In the nursing calf, the digestion of carbohydrates has an activity aminolitica reduced by the absence of salivary amylase, the action of the pancreatic juice increases as the age of nursing, as well as the development of the rumen, which contributes considerably to the digestion of carbohydrates and the production of VFA, which are absorbed into the wall of the rumen and carried through the bloodstream to the liver for liver glycogen synthesis advances [38], the maintenance of blood glucose and the deposit of fat from body reserve. The low capacity of nursing calves for starches derived from the poor activity of pancreatic

juice aminolítica. However, the intestine synthesizes appreciable amounts of lactase, which favours the use of sugars like lactose, glucose and galactose absorbed in the intestine [39]. However, lactase production descends gradually up to two months of age, if the consumption of milk remains the proximity of weaning. Sucrose is metabolized in the intestines by microbial action, the fruitful is not easily absorbed in the intestinal light so that its use does not is not recommended when calves suffer from diarrhea in the first weeks of life [11]. Carbohydrates sugars such as Xylose, glucose and galactose are absorbed easily into the small intestine in contrast to the fruitful, which is not absorbed in internecine mucosa. The use of glycine promotes the absorption of glucose and its transport in the intestinal epithelium. So this compound is added, like inulin in the rehydration preparations for diarrheal calves [7].

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DIGESTION OF FATS IN THE NURSING CALF

For dDigestion of the fat in the calf is very important, its contribution in the diet is close to the necessary 20 to keep the energy input required to meet calorie needs, in addition to promoting the absorption of vitamin D, E and carotenes during the weeks following the birth. The salivary pregástrica estearasa, is a lipase produced mainly by tonsillitis salivary glands after birth;, however, the estearasa shows more activity in the abomasum, due to the rapid passage of milk from the oral cavity to the abomasum, where it forms the clot of milk. To raise the age of the calf, pancreatic secretion increases and pancreas lipase acquires greater relevance in the breakdown of fat in the diet, which is previously emulsified by bile in the intestinal lumen. To decrease the secretion of the salivary lipase. F, fat intake also increases with the age of the calf [38, 68].

COLOSTRUM AND THE TRANSFER OF IMMUNOGLOBULINS

Nutrient-rich colostrum and provides neonatal passive transfer of immunoglobulins to the calf. Colostrum contains high proportion immunoglobulins 5 to 50mg/mL, with a proportion of IgG1, close to 90 of total immunoglobulin. Lesser degree IgA and IgM, in tato that the proportion of IgE is very low compared with milk [2]. On the other hand, its laxative effect is due to one increased rate of intestinal flow of the globulins and nutrients present in colostrum, which stimulates the movement of the intestine by the presence of colostrum, which leads to the expulsion of meconium and other toxins accumulated in the digestive tract, accumulated during pregnancy [11]. The expulsion of meconium occurs within 3 to 6three to six hours of born birth, after having consumed the calf colostrum, the mothermaternal stimulates the calf; the cow licks the perineal region for early expelling meconium, s. Some animals take to evacuate the meconium within 24 to 36 hours [39].

Bovine animals by placenta epiteliocorial type, are disabled for maternal fetal transfer igsf during gestation, calves are agamaglobulinemicos at birth, thus depend on the transfer igsf infant passive trascalostral. Through the continuous provision, it allows the passage of immunoglobulins in the small intestine, which remains permeable about 48 hours [11, 20].

However, the greatest capacity of intestinal transfer of immunoglobulins IgM and IgG isotypes is usually best during the first 3 three hours of life. Another important consideration

is that colostrum is rich in nutrients from high digestibility; : total solids, lactose, fat, immune globulin, vitamin A and D. Are There are also identified cytokines; ɣ-IFN, IL-6 and TNF-α lesser amounts. In addition to a high proportion of leukocytes present in colostrum: lymphocytes, neutrophils and macrophages [34, 48, 101, 138]. Because the neonatal agamaglobulinemia of the calf, it is necessary to provide colostrum to the newborn. T to ensure proper passive immunity from maternal antibodies [50, 97, 100]. Immunoglobulins, lysozyme and other nutrient-rich colostrum, provides, Furthermore furthermore, lactoferrin and lactoperoxidasas and complement fractions, identified as components of the innate immunity [155]. For its part, calostrales immunoglobulins IgG and IgM, coming from the serum transported without modification to the mammary gland acinis [42, 130], by a selective transport of blood to the mammary secretion without modification: the IgA is produced locally in the breast tissue and to a lesser extent because of local production of IgA by the lymphoid tissue of the mammary gland, recirculation of B cells, sensitized through [72, 78, 155].

Before delivery, within three to four weeks, the transfer of IgG and IgM in the blood into the fabric of immunoglobulin in the blood to the breast tissue is increased, to reach their maximum levels, some days before childbirth to occurs. Some factors like malnutrition of the mother at birth, due to the low energy protein intake, seriously affects the transfer of immunoglobulins and consequently the quality of colostrums [8, 95]. Other factors affecting the level of immunoglobulin calostrales are: short periods of rest to the drying of the cow, the age of the mother and the number of deliveries, the breed of cow and its programme of vaccination prior to delivery. If the lactation is premature before delivery to occurs, there is lost a significant amount of immunoglobulins, similarly the heifers have a poor in natural signing immunoglobulins colostrum: turn cows with a greater number of deliveries were they exposed to vaccination programmes prior to birth and received a dose of vitamin A and E two weeks prior to delivery, assessing the quality of this colostrum immunoglobulin and vitamin A-rich [8, 14, 130].

In the different dairy breeds at the time of delivery, immunoglobulins, albumin and total solids concentration decreases rapidly, reaching normal levels in milk not calostral after seventy-two hours of childbirth have occurred. While the concentration of lactose and water increases since that period [11, 131].

An optimal quality colostrum is considered when the total concentration of immunoglobulins is very close to the 50g/L in dairy cattle and a proportion close to the 65 gL in beef cattle [129]. Adequate intake of colostrum for the calf must be between 12 and 15 of its body weight, preferably administered during the next three hours of birth of the calf. To achieve a maternal transfer of immunoglobulins between 150 to 200 g is considered appropriate contribution levels of immunoglobulins passive immunity. When they are employees, lacto replacements of colostrum must orally provide 450 g per animal per 24 hours [36, 44, 45].

Colostrum is critical for the newborn to be the vehicle transfer maternal - neonatal of immunoglobulins, excellent source of energy and protein, in addition to the easy digestion of mammary secretion, essential contribution of vitamins and minerals to the birth, stimulate the bowel movement and increase the resistance and passive immunity of the calf front wings digestive and respiratory infections in the first weeks of life [6, 59, 92].

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The provision of high -quality colostrum is achieved by stimulating of humoral immunity in the mother, during the last six weeks of gestation, to produce a selective transfer of immunoglobulins against the diseases of importance economic and health in dairy herd and beef [8, 25, 64]. A commonly used strategy is vaccinating pregnant females against, diseases that cause of neonatal diarrhea caused by rotavirus, coronavirus, and *E. coli* F5+ [79]. In the same way, mananoligosacáridos dietary supply liquid of the calf, significantly reduces adherence to receptors on the cells of the intestinal epithelium. Several studies also denoted the importance of vitamin A levels in innate immunity associated with macrophages and neutrophils [137].

In the newborn animal, intestinal permeability allows the transfer of maternal - neonatal immunoglobulin present in colostrum, for the passage of immunoglobulins and other macromolecules of the intestinal lumen to the general circulation through the lymphatic route of lymphatic vessels of the submucosa to the thoracic duct, to be drained to the greater blood flow [20, 21, 23, 111]. Transluminal step of immunoglobulins in the former gut, occurs in the apical portion of the intestinal villi, to join receptor (FcRn) present on enterocytes in the intestinal epithelium, recipients are considered the major histocompatibility class complex heterodimeros I (MHC I), which are similar to those identified in the yolk sac of chickens [155]. In this way, present in the intestinal lumen immunoglobulins are transferred by pinocytosis, forming microvesiculas in the polar portion of the enterocito, which migrate to the basal portion of the cell to be exocitadas in the basement membrane to the lymphatic circulation of the submucosa [42]. Intestinal permeability is kept about 24 to 36 hours, then starts the closure of the permeability of the intestinal for immunoglobulins, to increase the secretion of abomasal hydrochloric acid, which activates the pepsinogeno, capable of degrading immunoglobulins, the decrease in the pH of the abomasum of between 6.5 to 6.7 to values close to the 3.0 and 2.5 the abomasum pH decrease occurs gradually from the first 6 six hours of born calves until after 24 hours, in addition to the decline of the anti-tripsico and protease inhibitors oros factor present in colostrum from birth until after 48 hours [13, 26];, however the Renin is not layered, affecting immunoglobulins as does acidic digestion once he it settles in the abomasum between 48 and 72 hours,; other changes occur the the intestinal villus due to the increase of mitosis in the crypt of the villi and the migration of new enterocytes which that replaced the intestinal epithelium, in addition to the increase in the activity of the diatasas and intestinal proteases, as well as a greater secretion of pancreatic juice, 72 hours after the calf being born [81]. For this reason, it is necessary to ensure the timely supply of colostrum of good quality between one and three hours of age of the calves;, later levels of immunoglobulins Sera reflects the quality and timeliness of the intestinal transfer of immunoglobulins from colostrum particularly of IgM and IgG1 isotypes. A failure of the infant transcalostral transfer occurs by: poor mammary glandular secretion in young heifers, low levels of immunoglobulins in colostrum from the mother and a poor intake of foremilk to birth [51].

The passive transfer of immunoglobulins through colostrum, dependent on intestinal permeability, the globulin and other macromolecules, which remains during the first 24 to 48 hours of life of the calf [51]. However in the first hour, of born calves acquire high levels of immunoglobulins of the types IgM and IgG1 among the top three and six hours of life after having consumed between 5 and 6 % of their body weight, and between 12 and 24 hours they should consume the calf between 10 and 15 of their body weight. The passage of immunoglobulins through the gastrointestinal tract is produced among other factors by the

absence of hydrochloric acid in the abomasum during the first 12 to 24 hours of birth, as well as the inactivity of the pepsinogeno in the absence of hydrochloric acid. On the other hand, only Renin acts on casein;, that during that period, the colostrum contains the inhibiting factor of trypsin and other proteases, which prevent the digestion of immunoglobulins in the intestine by the action of the pancreatic juice. For this reason, the immunoglobulins are not degraded in the abomasum by digestion acid and proteases intestinal during a short period of time [20, 21].

The rate of intestinal absorption of immunoglobulins, decreases dramatically after the first six hours of birth of calves. During the first 72 hours, there are detected in the serum of the calf high globulin levels;, however they quickly returned to the normoglobulinemia due to intestinal excretion and redistribution lymphoid of immunoglobulins [27], in addition to the loss by digestive and renal route in the early hours of born calves. For this reason, the levels of immunoglobulins of calves must be evaluated the first day and the seventh day of newborns with levels optimum sera of immunoglobulins between 10 and 12 mg/100 mL [27, 92, 108].

He It is considered a failure in the transfer of passive immunity, cColostrum through, when serum levels of immunoglobulins in the calf serum mg 100 less than 10 mL after 48 hours of birth. Levels below 5 mg/100 mL immunoglobulins sera, may represent a risk in the survival of the newborn calf [20, 21, 22].

USE OF MILK AND FERMENTED COLOSTRUM IN CALF PERFORMANCE

It is anticipated that the use of fermented colostrum is feasible, and milk of lower quality, stored at 20-22°C, will produce fermentation lactic acid generated from the use of lactose by bacteria, which acidify the product. It is necessary to prevent possible contamination of milk with other bacteria sporulated and fungi saprotrophs with appropriate hygiene measures [86]. Acidified milk can be stored for several weeks, to be used in the power of nursing calves, without showing significant differences in weight gain and body condition of the calf [49].

Fermented milks used in a volume of between 3 three and 4 four litres a day, after the first weeks of life of calves, has a positive effect on the upbringing of the replacements to directly reduce production costs, significantly improve digestion and reduce the frequency of neonatal diarrhoea [44, 78].

Due to the presence of bacterial strains such as *Lactobacillus acidophillus*, *B. bifidobacterium*, *Lactobacillus cremoris* and *Lactobacillus bulgaricus* in fermented milk [69]. T, the consumption of fermented milk is nutritionally beneficial by the level of protein, fat, minerals and vitamins from B complex, which they contain; they improve weight gain and the general State state of the animal [51, 117]. It has been suggested that the use of yogurt must start after 21 to 30 days of age of the calf [165].

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USE OF MILK REMPACERS REPLACEMENT IN THE NURSING IN CALF

The use of milk replacementmplacers on calf performance, is made pair lower food costs, when this is done based on the use of milk. To the be given in two doses of 2 to 3 liters each by the difference in weight and age of the calf [24], whose consumption is increased slightly between the second and third weeks of age [8, 9]. This volume of milk can be replaced by a substitute for good quality milk, administered at a temperature approaching 42 °C consuming consumed on a daily basis between 520 to 750 g of par necessary lactosubstituto prepare between 4 four and 5 five litters of diet liquid daily [15]. However, it is very important to consider the type of ingredients present in the lactosubstituto,; particularly with the quality of protein and dairy origin or other animal or plant source [96]. Tto interact this component with gastric digestion in the abomasum [165]. The protein can come from milk and dehydrated milk serum, serum blood and hydrolysates of high protein soy, whose digestibility tends to be for minor compared with the milk [30]. Natural soy used as not hydrolysed protein source may contain anti-nutrient and antigenic factors that can be developed conditions of food intolerance; antitrypsin factors of soybean, conglicinina and betaconglicinina and phenolic compounds [44, 45, 130, 137]. In the milk remplacersreplacement, the energy content may serve between 15 to 25 of the diet, close to the formula; Itit is mainly covered with fat milk and lactose, during the first three weeks of life. Digestibility and energy bioavailability of the milk substitute are affected by thermal conditions and animal welfare conditions [7, 80]. I in addition to the initial production of VFA for incipient ruminal fermentation and access to solid diet. In addition to the differences in the metabolic ratio of the fat and carbohydrates displayed by the calf during breastfeeding, essentially on the type of short chains fatty acids and unsaturated who have greater digestibility in the diet of the calf [42, 95].

The use of starch of wheat and rice, as the main energy source of the lactosubstituto, is controversial due to the differences displayed in usability by the nursing calf during the first weeks of life, related to the changes the prestomagos morphofunctional and activity of pancreatic amylase of the calf. Also that, there is evidence of the starch that is joined to the clot of milk, associating starch to the casein, causing postprandial hypoglycaemia [25, 70]. Another consideration in the use of milk remplacersreplacement, when diarrhoea is offering 50 of diet liquid and other, replace it with the solution electrolyte containing glycine, bicarbonates and phosphates to promote hydration in the period of diarrhea. Other authors have suggested that the use of probiotics containing *L. acidophillus* and Bifidobacteria favors recovery, if maintaining proper hydration and favourable environmental conditions close to the area of ambient comfort between 20 and 22°C [165].

USE OF ANTIBIOTICS AS PROMOTERS OF THE GROWTH OF CALVES

The use of antibiotics in the diet of calves predestete and post weaning used the: chlortetracycline, Oxytetracycline, Bacitracin zinc and Tylosin phosphate, to improve the weight gain and feed conversion efficiency [35]. In addition to decrease decreasing the frequency of digestive and respiratory infections. T, the food of calves in engordase uses tetracycline and par tylosin decrease incidence secondary bacterial infections and control of

liver abscesses in lot of fattening. Monensin sodium, Lasalocid, such as the Fosfolipol and the inoforos are used pair improve feed conversion, and reduce the production of gas in rumen. To modulate the ruminal fermentation to accentuate VFA production in particular of the propionic acid and counter the microflora bacteria acid lactogenicas and inhibit the production of lactic acid reducing cases of subclinical ruminal acidosis and reverse the incidence of lameness in the feed lot cattle. There is currently a growing restriction on the use of antibiotics as animal growth promoters. Due to the constant global concern, about this situation has generated great concern globally due to the development of antimicrobial resistance of the pathogens animal [50]. and their transfer of resistance to microbial pathogens in human population exposed to bacterial strains exotic from animal origin, ; for this reason, the European Union banned through the regulation of the 282198 Council of 17 December 1998, and suspended the use of antibiotic animal growth promoters by 2012, which includes Virginiamycin, Bacitracin zinc, Spiramycin and Tylosin [157].

To reduce the use of antibiotics as growth promoters and breeders of the animal health have been evaluated various natural alternatives, among which are the use of dietary acidificadors and modulators of ruminal fermentation, Prebioticsprebiotics, probiotics and symbiotic, celululiticas enzymes and oligosaccharides. Which has the most promising results cases to will be administered in the diet of the calves and the poultry for fattening [63, 125, 161, 162, 166, 167].

USE OF PROBIOTICS AND PREBIOTICS IN CALF FEEDING

In recent years, the concept of animal and human nutrition has changed, going to define as a balanced diet to meet the nutritional requirements of the individual. Today is, the concern is to the provide nutrients and other factors that promote the health and physical well-being of the body, for their optimal development. Under this premise, functional foods comply with consideration to provide nutrients for an optimum development, ; this aditivesadditives group includes: Probioticsprobiotics, Prebiotics prebiotics and symbiotic among other nutritional factors that contribute to the well-being and health of the individual. Probiotics and Prebiotics prebiotics to be administered in the diet, affect positively one or more organic functions that have an impact in a comfortable and healthy for the individual State state by reducing the risks of health of the host [154].

THE PROBIOTICS

Groups of probiotics, as functional foods, decrease the risk of intestinal disease, performing live microbial cultures supplied as bioregulators of microflora and immunity, prevent colonization and excessive development of microorganisms that cause diseases, even individually [19, 53, 58]. This protective of probiotics mechanism occurs by different mechanisms: competitive exclusion, occupied position, and local microbicidal activity among others. Similarly is the synthesis of proteins and organic acids with bactericidal action, as well as the synthesis of local defensins of the host in the intestinal mucosa [75, 82, 90], as that may encourage some bacteriophages. A condition of the characteristic of probiotics is beneficial

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microbial cultures to survive for some time in gut, remaining viable and active in the food during the intestinal transit [85, 92, 116, 118, 137, 141, 147]. Some of the most widely used probiotics belong to different groups of bacteria: *Lactobacillus ssp*., Bifidobacteria, Leuconostoc ssp., Enterococcus ssp., and yeasts as *Sacharomyces cerevisae* and *S. Bulardii* and others which that are administered in food and water, continuously contributeing to reducing disease reporting [1]. Depending on the age, bowel colonisation by beneficial crop to the intestinal microflora, can promote to existexistence of appropriate conditions of hygiene and animal welfare in production environments [5]. Other beneficial effects of probiotics supply is the production of organic acids in short chain that Act act regulating intestinal microbiology, help tos regulate the metabolism of fatty acids and cholesterol, bile secretion, as well as contribute to the absorption of ions of Mg^{2+} and Ca^{2+}, faith and inmunomodular the immune response. However, different authors agree that the main effect of probiotics is the inhibiting reactions toxic metabolite toxic like the nitrosamida, indole and skatol among others. In addition, it to stimulatese enzymatic reactions that allow eliminatinge toxins and promote promoting the synthesis of vitamins and cofactors, which may be deficient in a diet [4].

THE PREBIOTICS

The prebiotics are non-digestible food components, which promote the growth of beneficial bacteria in microflora. Unlike the symbiotic constituting an Association of probiotics and Prebiotics formulated to ensure the implantation and survival of beneficial bacteria supplied in the diet [8, 99, 109]. T, the products considered Prebiotics include the: oligosaccharides, glucoproteinas and fructoligosacáridos, Mannan, enzymes and organic among others than to be supplied in the diet acid liquid from a baby animal;, they favor the development of the microflora protective against intestinal pathogens [28, 29].

Dietary fiber in foods (FD), is currently regarded as ingested polysaccharides in dietary not digested by endogenous enzymes secreted by the individual. W, which may increase the VFA production, in the colon and the blind, stimulating wings colonic epithelial cells mainly due to increased blood flow in the mucosa and submucous colonica [41, 55]. Has It has been considered that the fund contributes to acidifying the medium in the colon and the cecum of the monogastric and lactating ruminants to reduce the bacterial population of *E. coli, Salmonella ssp* and *Streptococcus fecalis*. The use of lactose, mannose and acid propionic contributes to reducing colonization by enteric pathogens in birds and pigs, ; it is possible it also could have a similar effect in nursing calves [51]. Other oligosaccharides avoid bacterial adherence to epithelial cells of the intestinal wall and decrease of *E.coli* in the calves. Some oligosacaridos are used by the microflora as growth substrate. Oligosaccharides of soybeans, fructo-oligosaccharides and galacto-oligosaccharides are par employees induce the colonization of bifidobacteria and B - AL. D, due to the great variation that may have these bacteria may have on changes in diet and fasting periods [55, 82].

The Prebiotics prebiotics affect the intestinal microflora modulate intestinal microbiology, promoting the increase of anaerobic bacteria beneficial to the host. The main advantage of the Prebiotics prebiotics is the favour induction of microflora and their

bioregulation through the provision of non-starch polysaccharides, oligosaccharides and hydrolysed starches [82].

MIXTURES OF PROBIOTICS

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Probiotics are considered in some regions as zootechnical additives, which includes in its formulation as bioregulators desirable microorganisms and enzymes. A, as well as other nutritional compounds that promote bowel activity of probiotics such as: calcium, sulphate magnesium, citric acid, ascorbic acid, silico aluminate of sodium, oligosacaridos. In addition to dried crops of bacterial strains, fungi and yeasts tested in its probiotica activity. The most commonly used in the feeding of ruminants are to *Lactobacillus acidophilus, L. lactis, L. casei, Streptococcus diacetylactis, Bifidobacterium bifidum, Sacharomyces cereviciae, Bacillus subtilis, Streptococcus faecium* and *Aspergillus oryzae* [67].

The use of probiotics in ruminants are from the species Lactobacillus ssp, and Enterococcus Bacillus subtillis, and among the fungi *Aspergillus oryzae* and Yeast *Saccharomyces cerevisiae*. As pProbiotics additives, are viable cultures of microorganisms, as no nutritional food supplements administered to animals, ; it has a beneficial effect by modulating the microflora and stimulating immunity [48, 119, 120].

Probiotics, also known as microbial additives, are prepared commercially, containing viable micro-organisms forming colonies (UFC) units estimated between 1 to 2 x 10^8 and 4 x 10^{11} and are used as additive as a prebiotic UFCg. Although the doses that can be administered to animals are variable depending on the type of microorganism, it has capacity for adaptation in the digestive tract and or his its intestinal transit. A, as well as the production stage in which there are animals [48, 99, 100]. In other countries, their use and dose supply this legally regulated; there is an establishment of a minimum and a maximum of UFC/Kg of food prepared in the diet. It should be noted that the effect of probiotics is usually better in the early stages of the life of production animals; in the first weeks of life and later after weaning, axial as well happens tensiónales periods of production, management, diet and unfavourable climatic conditions [94, 103]. It should be noted that the supply of probiotics should be constant through food or drinking water; already the multiplication in the digestive tract may be compromised. The addition of symbiotic mixtures seems more desirable as microbial additives in animal diet of the lambs, calves and pigs, improving food intake and daily weight gain [50, 71, 76]. In ruminants of different species and stages of production, the *S. cerevisiae* is often the most versatile and easy of supplies as well as the Mannan-glucans as promoters of production and growth [71, 119, 120, 139].

PROBIOTICS AS LACTIC ACID BACTERIA USE

Lactic acid bacteria (AL-B), are considered an important group of probiotics used in human nutrition and animal nutrition [141], which derived from the fermentation of milk, using the lactose in milk as a source of carbon, pair create lactic acid fermentation product [118]. The AL-B therefore constitute a wide range of healthy bacteria par food and biofeedback in the intestinal microflora. The AL-B constitutes a functional group of

Gramgram-positive bacteria acid lactic producer, not pathogenic bacteria, not useful for the fermentation of food toxigenic. The Group AL-B include: *Lactobacillus species, Lactococcus ssp*, and *Streptococcus thermophilus* [116, 118, 127].

Lactobacillus acidophylus, in addition to producing lactic acid, acidifies the environment contributing intestinal protection front wing wings pathogenic bacteria. The bífidobacterias protect the large intestine, favoring the acidification and proliferation of Lactobacillus ssp, in addition to producing significant amounts of b vitamins, u Unlike the *Lactobacillus bulgaricum*, which has a short stay in the intestine, but also contributes to the modulation of intestinal microflora and of local immunity [4, 5, 48]. Group AL-B, includes the *Lactobacillus ssp*., *L.casei* and *L. acidophilus*, commonly used as probiotics [116],; bacteria must be protected from the step by the acid environment of the stomach via micro encapsulation with algilato, yucca and gum Guar t. This procedure ensures a greater number of viable bacteria present in the digestive tract [145].

The genus *Bifidobacterium* is not fermented food, so it is taxonomically different acid bacteria lactic, by what is not usually grouped among the AL-B. Certain bacteria apatogenas such as *E. coli*, and some spore formers, and yeasts used as probiotics do not belong to the Group AL-B [124]. Yeast such as *Saccharomyces cereviciae* and *Sacharomyces bulardii* [1, 2, 148, 149], cultures act stimulating bacterial, mainly from the bacteria growth celulolíticas, and those that use lactate as a substrate, improveing the conditions of the ecosystem rumen with benefits on animal nutrition, favoring a speedy recovery of the negative energy balance typical of the initiation of breastfeeding, although it has been observed that there are differences metabolic and functional among strains of yeasts employed as probiotics in animal feed [119, 120, 149, 150].

USE OF GLUCANS IN DIET OF CALVES

The Mannan glucans (MOS), added to the diet prevents microbial colonization, affecting the adhesion of bacterial lectin to carbohydrates present on the surface of the intestinal cells. This action reduces the colonization of the digestive tract by that cause neonatal diarrhea pathogens, which are then excreted in the feces. Then the mechanism of action of the MOS is different to from the antibiotics used as promoters of animal growth on bacteria in the intestine. The main action of the MOS is reducing intestinal bacterial colonization by pathogens, while avoiding microbial resistance to antibiotics [33, 34, 144, 146]. Pathogenic bacteria are joining the manosas located on the outside of the host intestinal cells, fermented by pathogens. This Uunion mechanism present in the pathogenic enterobacteria by type I fimbriae are Mannose-sensitive, as opposed to the pathogens that are Mannose-resistant [47, 48].

The MOS have been considered immunomodulators from the immune response exacerbated during periods of adaptation of young animals. S, stimulating innate immunity by phagocytes and immune mechanisms of adaptive immunity that interacts with the APC and the LTh1 cells. The presence of cytokines and wall bacterinabacteria, stimulate chemotaxis and phagocytosis of pathogens present in the intestine of the host. By stimulating the activity of local phagocytosis in the internecine mucosa, this also produces the secretion and recirculation whole liver of IgA in intestinal mucosa of the jejunum, ileum and colon. In

addition to the elevation of IgG1 immunoglobulin in the blood serum local, there is high activity mitogenica of LB and LT. It is possible that the MOS, interact with the intestinal epithelium cells M, to be transported to the Peyer´s plate for the subsequent secretion of IgA and IgG1 and IgG2, Peyer. Has It has been considered that a daily dose of 1.5 to 9 g for animal dietary is adequate par to maintain balance intestinal microflora of calves and improve daily weight gain [152].

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USE OF ORGANIC ACIDS IN RUMINANT DIET

Organic acids are part of animal tissues,; they are intermediate products of some metabolic cycles, and some of them are also produced in the digestive system of animals during fermentation processes. The AO are used regularly in the diets of monogastric;, however in ruminants, they only have experience with the use of the malato and fumarate as no nutritional additives [93, 119]. The AO are commonly used as an additive in monogastric feeding, but their use in ruminants is limited. The mechanism of action of organic acids (AO), is not entirely known; however, its main effect is attributed to acidification of the intestinal microenvironment that modifies the microfloras intestinal and affects the enteropathogens intestinales [31, 33, 34]. Unlike the monogastric ruminants, the effect of the AO is attributed to the local action of the AO and its related compounds capable of modulating the fermentation ruminal and VFA production [112]. Main compounds used as AO and its related products in the feeding of ruminants are organic acids is a such as: malic and fumaric as additives no nutritionals [33, 106]. The required concentrations par in modular fermentation ruminal and biomass are small, micro-organisms that can ferment between 7-10 mM malato and fumarate between 12 and 24 hours. Therefore, they do not generate residues in products of animal origin that affect quality and safety of food derived from ruminants production [32]. Likewise, some acid lactic utilizers bacteria as *Selenomonas ruminantium* population significantly increases, and also is able to ferment carbohydrates Monosaccharides and disaccharides, oligosaccharides to produce acetate, propionate and lactate [34, 52].

It has also been observed that the malato reduces the frequency of the rumen Parakeratosis and increases the size of the ruminal papilla. Current studies indicate that the use of the AO in ruminants reduces the production of methane, considered an effect of gas greenhouse (GEI) of agricultural origin [32]. This effect is attributed to the fermentation makes the *S. ruminantium*, which is metabolized to the malato and fumarate to succinate and propionate production, in turn reducing the generation of hydrogen and methane [12, 17], to administer to an 11.7 of fumaric acid in the diet of cows in confinement, causing a reduction in methane between 50 and 70 % at the same time decreasing the concentration of hydrogen in the rumen and lowering as well the amount of available hydrogen to form methane in animals with a high ration concentrates [31]. *S. ruminantium* has been studied as one of the bacteria ruminal of greater importance in ruminants in confinement undergoing diets high in concentrated and rich in energy; the S.ruminantium constitutes the largest proportion of ruminal bacteria in animals adapted to high production diets. This bacterium has the ability to ferment different types of carbohydrates; : fruitful, glucose, Galactose, maltose and lactose. In animals adapted to the diets of high- energy content, of the S. ruminantium uses the lactic acid to produce propionic acid. It is possible that administration malato and fumarate, also a

generating microbiota of *S. ruminantium*, helps control acidosis subclinical rumen in ruminants in confinement of fattening [33, 34].

USE OF ENZYME EXOGENAS IN THE FEEDING OF RUMINANTS

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Exogenous enzymes used as no nutritional additives (EEA), in the feeding of ruminants is interest insofar as they can improve the rumen digestibility and increase production in ruminants [16, 113, 166, 167] t. To encourage the use of cellulose, hemicellulose and cellobiose, for the production of VFA in the rumen. T and to be downgraded the structural components of the wall and the cell contents b. By enzymes such as: chitinases, xilanases, β-glucanases, pectinases, taninases, proteases and amylases [134]. The source of these enzymes comes from bacteria, yeasts and fungi; *Lactobacillus acidophilus, L. plantarum*, *Bacillus subtilis* and *Streptococcus faecium*. *Saccharomyces cerevisiae*, *Humicola insolens* and *Thermomyces amiginosus Trichoderma reesei* and *Aspergillus oryzae* [10, 16, 17]. The activity of the enzymes is classified according to their action on substrates of vegetable wall [32, 34, 99, 100]. Enzymes are classified as: cellulase, xilanases, proteases, pectinases and amylase, β-glucanases, phytases and tanases [57]. Food ruminal digestion is a complex process involving numerous enzymes that contribute to degrade the cell wall of plants, starch and proteic nitrogen and nitrogen no proteic a. Are considered ah that the use of the EEA and its compounds of commercial use, depend on the type of enzyme, substrate and conditions of cultivation for industrial production [114].

MECHANISMS FOR INTERACTIONS OF PROBIOTICS AND THE HOST

Probiotics are different on the health of the individual actions through different mechanisms of action [48, 102]. A basic mechanism is the acidification of the intestinal content, which contributes to the bioregulaciòntion of the intestinal flora. Other more complex mechanisms are mostly immune modulation in the intestine and the secretion of substances that inhibit the growth of pathogenic micro-organisms; to decrease the availability of nutrients for pathogenic bacteria, joining competitively to the intestinal mucosa, preventing the action of pathogens [53, 57, 83, 88, 89, 156].

Induced inmunomodulaciontion is produced by the movement of IgA to the presence of rotavirus and *E. coli*, as well as facilitated by the capture of antigens soluble in to Peyer's patches [28, 29, 104]. To contribute to the modulation of inflammatory in the intestines process affected by pathogens and other enteropathies are released Cytokines and stimulate LTh2 and NK cells on cells swollen and precancerous in experimental studies [55, 67, 74];, however the latter effect has not been evaluated in calves and humans [89, 151].

Another effect commonly accepted in human nutrition is the increase in the hidrolases, to join the cholesterol reducing their levels with a hypocholesterolemic effect. To increase the synthesis of short-chain triglycerides, they inhibit the production of cholesterol and promote redistribution of the plasma to the liver. Deconjugation of bile salts, cholesterol is not recirculated and is subsequently used for the synthesis of new bile acids. Although many mechanisms are derived from experimental observations in animal models of laboratory [83],;

there are currently few studies showing evidence of clinical studies *in vivo* [65, 66, 84, 105, 107, 110, 115, 144].

However, it has been suggested in the gut probiotics stimulate the immune system, particularly the Bifidobacteria in the intestinal transit and , stimulate local production of IgA, which neutralize intestinal pathogens, like lysozyme and present in the intestinal mucus [58], defensins. Earlier studies had pointed out the influence of the AL-B [19, 103, 106];, some of the bacteria are surviveaival the chemical barrier passage by the stomach and them they expressed in the intestine his the bioregulation on microbial biomass and their their inmunomodulation of the innate and adaptive intestinal immune response [98, 110, 148]. Currently, it is known that some strains of probiotics delayed hypersensitivity, the production of IgA and IgG2 antibodies, as well as the functional activation of macrophages and other antigen presenting cells [APC] [43]. Also shown in field studies is the usefulness in the prevention of enteric, respiratory and digestive diseases during lactation in animals and man [61, 62, 150].

In innate immunity, the first line of defense comes from natural barriers, such as the skin and the mucous membranes, which decrease the risk of infection. Similarly, phagocytosis, physiological and chemical barriers contributing to reduce microbial colonization in herthe body [153]. Although the mechanism of adaptive immunity more known by the administration of probiotics is the reproductive and intestinal mucosa secretory immunity, both in the mammary gland and vaginal level, noted as the defensive mechanism against entericas diseases. Through secretion and circulation both IgA and LTh1 that react against the antigens present on the mucosa and produce the immuno exclusion [132, 135, 144].

It is widely accepted that probiotics affect the intestinal ecosystem by encouraging the mechanisms of the innate and adaptive, immunity as well as the competition set out with microorganisms of probiotics and the bacteria of the gut flora or competition with enteric pathogenic potential [125, 127, 140], w. Which consequence is the reduction of the incidence and severity of diarrhea, enhancement of digestive activity and reduction ofe inflammation of the intestine. Although there is a need for more scientific evidence to support their clinical effects under conditions of field both in animals and man [48, 121].

The intestine is the related to the most important immunological function of the agency, to contain a large population of immune cells, their immune response is complex, which implies different phases in which is includeds the identification of the Antigen, neutralization and destruction thereof; and feedback [72]. The interrelationship of the bacteria probioticas and host is established by a symbiosis which that influences the local immune response through the present in the small intestine lymphoid structures, in the colòn and the blind probioticas bacteria used substrates of the environment to produce VFA contributing to acidify the medium and to countering pathogenic bacteria. Some batteries use as probiotics such as L. acidophylus-LAB1, and the Bifidobacterium bifidum are susceptible to be phagocytosed by neutrophils and macrophages in man, than to be processed by the macrophages as APC cells contribute to stimulate local secretion of IgA and Th1 lymphocytes [28, 29].

It hHas been raised that the AL-B they colonize the intestine and can survive the passage through the digestive tract, by joining the epithelium modify the local immune response of the host in studied animal models. Protection against intracellular pathogens of the B - AL may be associated with the activation of endothelial lattice system when they constantly ingest fermented milks [73, 83].

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MUCOSAL IMMUNITY IN CALF GUT

Local secretory immunity depends on immunoglobulin IgA, which rapidly binds to receptor glycoprotein called the secretory fraction (J), present in the basolateral enterocyte portion. The IgA component and J fraction covered with clatrin be internalizing by vesicles endociticas to the enterocito to her the sheets, to its own recircular to the mammary gland [72]. Lymphocytes circulating in the intestine (GALT), are paramount in the gut mucosa immunity. The lymphocyte in Peyer's activity and the mesenteric linphlymph nodes are essential for inmmunoestimulation of local T cells interacting with the presenters of the Antigen (APC) cells [138], r. Recirculation of intestinal lymphocytes is a phenomenon that occurs in animals after birth;, these cells have the ability to differentiate between antigens after birth by the Lymphocytes B captureing antigens circulating and the Lymphocytes T to capture antigens from the cells APC, developed from the report produced by secreting immunity [94]. For recircular the IgA across the entire liver system. Secretory immunity is stimulated in large part by the intestinal microflora, which increases the ability to recognize different antigens in the intestine [72, 155].

It has been observed in humans that the administration of *B. bifidum* and *L. acidophylus* stimulate the regional presence of lymphocyte B cells, circulating from Peyer's in the intestine, where there is inflammation of the bowel. The administration of a mezcal probiotics containing L. casei and Bifidobacterium longum and l. acidophylus causes the local IgA secretion and the cytotoxic activity of t cells. It has also shown that there is a local increase of Th1 lymphocytes and NK cells. In addition is and the secretion of cytokines: IL-2, IL-4, IL-6 and ɣ-IFN when the host are receiving *L. acidophylus* and *B. bifidum* [73, 124]. Therefore there is strong scientific evidence to support the use of probiotics with bacteria (b) - to the and Bifidobacteria as immunomodulators bioregulators and local immune response of the intestinal microflora [40, 41], highlighting the following benefit in the Adaptive immune response; activation of macrophages premises as APC cells to lymphocytes Th1, modulation of the immune response through cytokines, reduction of food intolerance and hypersensitivity reactions and m. Modulation of intestinal microflora. In the process of digestion, the production of water-soluble vitamins, acidification and reduction of the atmosphere for the intestinal microbiota, increase the digestibility of nutrients [61]. Other additional benefits in innate immunity are the increase in the secretion of intestinal mucus and secretion of defensins and pathogens locally, as well as improving the functionality of the intestinal barrier. The increase of the symbiosis of the probiotics-prebioticos and the intestinal microflora competing with the pathogens opportunists that contributes to modify the production of substances toxic by flora [122, 128].

ACTION OF PROBIOTICS IN DIARRHEAL PROCESSES

The administration of B - AL to calves after birth, reduces the frequency of diarrhea during lactation period. P, particularly when diarrheal table is associated *Escherichia coli* by reducing their occurrence and severity of enteric colibacillosis boxes [123]. It has been shown the antagonistic effect of the the B -AL envelopes the action of different pathogenic types of E. coli. H and has been considered that the treatment of acute and chronic diarrhea with *L.*

casei and *L. acidophylus* is beneficial, as well as preventing the infection of other individuals with pathogenic enterobacteria. In addition to producing a serico increase in the levels of antibodies against the bacteria enteropatogenas front infection active in the population, which shows the interaction of immune mechanisms and the presence of the probiotic that stimulates immunity in the gastrointestinal tract [73, 76].

The addition of probiotics in young animals is used to regulate the dysbiosis of intestinal flora, by favouring the normoregulaciontion and even desirable bacteria increased digestive function especially in calves, lambs and nursing pigs. With the inclusion of probiotics, as well as avoiding problems such as septicemic and enteric colibacillosis, expect better weight gains and better food conversion both in the monogastric, prerumiantes and birds in the first weeks of life [143].

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USE OF PROBIOTICS IN CATTLE

Probiotics are also considered additives that are not nutritious, which can be administered in mixtures of probiotics and pPrebiotics, in other cases of symbiotic, probiotics and modifiers of ruminal fermentation, probiotics and enzymes [71, 161, 162]. W, which can be developed from crops of *Sacharomyces cereviciae* as viable yeast and derivatives of yeast killed as the Mannan glucans produced by hydrolysis of the wall of yeasts. W, which stimulate the symbiosis of microflora rumen, the synthesis of cobalamin and thiamine among other digestive functions [162]. Has It has been considered that *S. cereviciae* strains 1034 and 1026 when was administered with other prebiotics and minerals, increase the production of VFA, improve production of microbial protein, celulolitic activity and the use of nitrogenous sources provided in the diet of the animals that consume forages and get diets based on concentrates and forages. To provide between 5 and 10 g of yeast can maintain a population of 2 to 4 x 108 UFCmL of ruminal fluid [139]. It is possible that one of the major effects of probiotics based on the B - AL is to regulate the production of lactate by *Lactobacilli* in the compartment endoplasmic-ruminal when given diets high in carbohydrates, because *Lactobacilli* stimulates colonization of bacteria consumers of the acid lactic and synthesizers of propionic acid [33, 34].

The rearing of calves is one of the most important challenges in livestock production, to be the starting point for different purposes of the company, both for the production of milk as the meat [50, 56]. The use of probiotics in feed calves' feed provides nutrients and digestive enzymes, which promote growth and reduce the mortality of calves at weaning [143].

The use of milk, acidified with AL-B, it has been suggested to replace antibiotics as growth promoters. To maintain a positive balance of microflora rumen and intestinal, occurs the eElimination of digestive pathogens. Nursing calves and juveniles of other species is are stimulated the microflora when given different strains of probiotics as: *Lactobacillus acidophylus*, *L. bulgaricus* and *Streptococcus thermophylus*. The use of probiotics in animal husbandry has shown countless advantages, because it decreases the cost of food, increasing the capacity of assimilation of protein, energy and minerals. There is a greater proportion of bífidobacterias in the gut; the gut contents acidifiesacidify, decreasing abnormal intestinal fermentation stimulated the production of mucus in bowel and an increase in intestinal peristalsis [146].

Crops such as *S. cerevisiae* yeast stimulate the microflora rumen celulolitica and increase the capacity of the utilizadoras bacteria of the lactic acid. P, producing positive results in digestion ruminal on celulolitica activity and VFA production, the proportions of bacteria celuloliticas biofeedback and aminoliticas bacteria. Another effect is promote the transit of the nursing calf to a ruminant young [139].

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CONCLUSION

1. The challenge now facing dairy farming and beef is to maintain a responsible and sustainable production. N, which is necessary to maintain the productivity and competitiveness of the companiesy in the food market.
2. The use of nutritional additives adaptogenos emerges as an important alternative for livestock production as well as; employees as adaptogenos in the upbringing and development of nursing, prerumiant calves at weaning and development.
3. The adaptation of the newborn calf to the new environment of production and breeding is favored with the use of probiotics, Pprebiotics and symbiotic. It has productive advantages by inducing desirable ruminal fermentation and, the bioregulatcion of formulated probiotics with crops of S. cerevisiae and microorganisms AL-B to, simple and associated with other symbiotic. Experimental studies and fields they underpin a beneficial effect in animal production to the regular microflora and the inmunomodulaciòntion during the upbringing of young animals.
4. The use of prebiotics and symbiotic in livestock currently this is part of broad development and research. To and demonstrates a potential usefulness in sustainable and responsible production related to food safety and environmental impact in the production of greenhouse gases from agricultural origin.

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Chapter 13

IMPLICATIONS OF THE USE OF CLENBUTEROL HYDROCHLORIDE IN BEEF CATTLE

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ABSTRACT

Today, ranchers and producers intending to improve performance results in conversion and maintenance efficiency in various animal species (cattle, sheep, pigs and poultry) resort to the use of substances such as hormones, anabolic and additives, among others, one of these being the clenbuterol hydrochloride (CCL). Therapeutically, the CCL is a powerful simpáticomimético with great affinity for bronchial area β2 receptors, so the most important effects are bronchial dilation, elimination of bronco spasm, stimulation of the vibration of the bronchial Cilia, facilitating coughing and increasing lung ventilation with prolonged effect. On the other hand, oral administration of CCL modifies the growth of muscle mass and decrease fat accumulation. Under physiological conditions, the growth of skeletal muscle is the primary result of hypertrophy, an increase in muscle protein synthesis and a decrease in muscle protein degradation. This substance may increase blood flow to certain areas of the body, allowing the process of hypertrophy in skeletal muscle to carry larger amounts of substrates and sources of energy for protein synthesis. Another action of this compound is the decrease in the amount of fat in the channel, an anabolic effect that is achieved by increasing ten times its therapeutic dose (0.8 μg/kg). However, this has brought impact to public health, so its use in species to supply this banned in clinical cases of poisoning in humans, has been reported that the main signs are vomiting, muscle tremors (in some cases numbness of limbs), headache, increased blood pressure, and tachycardia, which may end in death by heart failure. So using other alternatives (such as the elaboration of diets with use authorized supplements) in animal production and in particular in cattle must be evaluated in order to determine effects on public health.

INTRODUCTION

The bovine cattle ranch has shown important changes in the last years—associated the automation—that have allowed to, at present, obtain efficiency levels that had been impossible at other times. Thus, the farming production is ligature to the use of productive tools; therefore, the growth of the investigation and development have allowed the massive diffusion of these tools that are of fundamental aid to the producers [42, 52].

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Within the important aspects in the production animal, the security is due to consider nourishing, but at the same time as the farming foods are free and innocuous, those guarantee the feeding of all the consumers. At present, many producers within their production systems make use of a great amount of substances that are applied, added or included within the feeding of the different productive species with the interest of improving the productive parameters—reproductive, neglecting to a great extent the innocuity aspect that implies the presentation of problems in public health [40].

In the last years, the problem of massive poisonings has appeared in European countries (France, Italy and Spain) by the use of Clenbuterol Hydrochloride (CCL), which is a substance β-agonist (pharmacologically active compound that acts improving the retention of nitrogen compounds, is chemical agents that turns aside the energy and the nutrients of foods and the fat reserves of the animal towards the protein synthesis and muscular incorporation) in the feeding of the cattle of become fat, since these increase the weight of the animal due to the increase in the muscular mass and this way to obtain animal with a low fat content [9, 24].

In bovines, in low doses (considered like promoters of the productive yield), the CCL induces an increase of the sanguineous pressure, transitory increase of the cardiac frequency during 24 hours approximately, increase of the metabolic rate and has inquired that increases the rate of giving up. They had not documented the effects of an overdose of clenbuterol in this species, but they do not have to differ more from the previous thing than in its magnitude. To promotional doses of the production or superiors, the problem of the illegal use of clenbuterol concentrates mainly on the risks that the product ingestion represents for the consumer of contaminated origin animal with this one [1, 50].

In yearling calves, the level initial tip of CCL after a 5 dose of μg/kg/day, considered like promoter of the yield, went to 0,5 ng/mL two to seven hours after the treatment. After 21 days, the level tip probably arrived at 1,1 ng/mL, four hours after the dosage, due to the fact that the denominated steady state with a light level of accumulation has been reached. In nursing cows dosed a chronic way with clenbuterol at the rate of 5 μg/kg every 12 hours, there was reached a maximum plasmatic level of 5-5,5 ng/mL at five to seven days after its administration [13, 50].

In public health, the potential problem must be a question of the concentration of this one in ingested foods and not be a cumulative genomic toxicity. The effects derived from the product ingestion contaminated with CCL are muscular sleepiness of the hands, tremors, muscular nervousness, and headaches. In acute-extreme overdoses, not derived from the product ingestion with residues but a product of pharmaceuticals, an accidental overdose of the human line that contains clenbuterol, one accentuates the tachycardia, the sleepiness, the nervousness, the tremors and can have necrosis of the myocardium by diminution of the perfusion generated by the shortening of the diastole, the stage in which the irrigation of the myocardium by the coronary ones is carried out [4, 13, 50].

Doubtless for the producers, the inclusion of CCL in the diet of bovines has generated important gains economically; nevertheless the problems in public health and in health animal require that specialist in the sector veterinary health, doctors and epidemiologists work together to safeguard the collective health. And the SAGARPA at Federal State level also must maintain operative of monitoring and the control, for the eradication in the use of this substance. The objective of the present work is to determine the levels of CCL in samples of urine of bovines sacrificed in three signs of the State of Mexico so that it is oriented to all involved in this activity to take the precautionary measures in animal control in which use has become of this substance [9].

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CLENBUTEROL HYDROCHLORIDE (CCL)

Clenbuterol is a synthetic derivative pertaining to a class of analogous medicines' physiologically to the adrenalin that has the capacity to interact with adrenérgicos receivers, generally of the type ß2 (β-agonist) [27].

Adrenergic physiological agonists β- are the norepinephrine and the epinephrine. First it constitutes a catecholamine of the group of the fenetanolamins; it also is considered like a neurotransmitter of the likeable nervous system that biosintetiza from the tirosina and is circulated in the serum in relatively elevated concentrations. Second of the same group secret investigation in the adrenal marrow is synthesized, and it circulates in smaller concentrations than the norepinephrinea in the majority of the mammals; but in stress situations, it responds in greater proportion than the norepinephrine. The norepinephrine is more selective of receivers α and the epinephrine acts on both, with greater selectivity by the receivers β, but with a more dominant effect α. The physiological answers take place when these β-adrenergic agonists are united to the receivers and for the case that occupies to us, to the β-adrenergic receiver [27, 39, 55].

They are adrenergic organic molecules β- that are united to the ß-adrenergic receivers, giving rise to the agonist-receiving complex that it as well activates to protein GS [2]. The subunit α of active protein GS to the adenilato-cyclase enzyme that produces monophosphate of cyclical adenosina (AMPc) is one of main molecules of intracellular signaling. This molecule produces its effects when being united to the regulating subunit of cinasa protein To, to release the catalytic subunit that fosforila to a good number of intracellular proteins. These proteins have vital functional papers for one varied range of functions that are going to allow the entrance of Ca++ to the cell, until mediating the key protein synthesis for the cellular operation [14, 28, 43, 45].

Other possible mechanisms include the affinity of the agonist by the receivers β, the connection of the agonist-receiving complex to the trasductor system of signals, factors that influence the transport from the drug to the sites of the receiver; this as well as determining by each species since each of this has a limited number of β-adrenergic weave receivers white that reduce the answer to the agonist [27, 48].

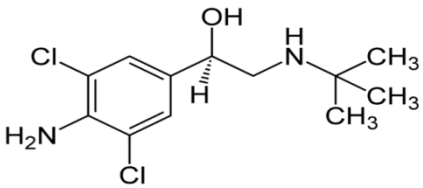


Figure 1. Chemical structure of the molecule of clenbuterol.

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The clenbuterol is white, anhydrous, very soluble in water and has a highly stable dust to room temperature; its fusion point goes of 174 to 175.5°C [17, 46]. It has one structure chemistry related to catecholamine's able to interact with adrenergic receivers, generally of the type ß2. Chemically amino metil alpha butilamino T 3 is amino 4, 5 diclorobenzil alcohol. It owns an average life of prolonged action, with the particularity of being able to store in liver and kidney and metabolizes by means of glucoronics reactions of N-oxidation in hidroxyclenbuterol and conjugated [12, 14, 30]. (Figure 1).

Therapeutically clenbuterol is a powerful simpaticomimetics with great affinity by the receivers β2 of the bronchial zone, which is a reason why the most important effects are the bronchial expansion, eliminating the rough spasm, the stimulation of the vibration of the bronchial cilia facilitates the expectoration and the increase of the pulmonary ventilation with prolonged effect [10].

For bronco spasm, bronchitis, bronquiolitis, pulmonary disease chronic obstructive and acute and chronic pulmonary affections, give good results to the being administered jointly with antibiotics, and prevent respiratory allergic reactions as well. Thus it offers the benefit to induce a well-known broncodilatacion in the man to dose of 10, 20 and up to 40 μg/adulto, and in horse bovines and the dose is of 0,8 μg/kg [7, 10, 38].

But within many nutritional additives used in the feeding animal, we found the CCL, which to dose of ten times superiors to the therapeutic one, presents/displays an anabolic action, favoring the protein synthesis and diminishing the fat [22, 41].

The oral administration of some synthetic β-adrenergic agonists modifies the growth by increase of the muscular mass and diminution of the fat accumulation [15, 43]. The β-adrenergic receivers are present in the majority of the cells of the mammals, although the distribution of the subtypes (β1, β2, β3) and the proportion of each vary between weaves and species [27]. Generally, β1 predominates in the heart, stimulating their inotropism (contraction force) and in the intestinal smooth muscle, inducing relaxation, whereas β2 is located in the bronchi and uterine muscle to them, inducing relaxation in both cases [34, 43]. Evidently, the magnitude of the phisiopharmacologic activity of an agonist or β-adrenergic partial agonist will depend on its denominated intrinsic activity in the receiver and distribution in weaves white [46].

One of the most obvious effects of the oral administration in the cattle, pigs and bovines, is the increase of the muscular mass [46, 49]. In physiological conditions, the growth of the skeletal muscle is the primary result of a hypertrophy, and it is clear that an increase of muscular protein synthesis and one diminution in the muscular protein degradation or a combination of both produces increase of the muscular mass [54]. Thus it can increase the sanguineous flow to certain regions of the body, which allows to the process of hypertrophy in the skeletal muscle when transporting major amounts of substrates and power plants for the protein synthesis [11, 46, 48].

Another one of the main actions of the CCL is the diminution in the amount of the fat in the channel. One has demonstrated test-tube to the degradation of triacilgliceroles in adiposities and the inhibition of the fatty acid synthesis and of triacilglicerol, the weave of the animal presents/displays an increased lipolitic activity, a diminished lipogenic activity, or both [20, 25, 26].

The elevation of the plasmatic, not etherify, fatty acid concentration after the administration of clenbuterol confirms the lipolitic activity that happens in the adiposities' [18, 25].

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The use of this compound in high doses can cause a deposit in different organs, mainly in liver [5]. This accumulation can bring about poisoning in the people who consume this weave [47].

The problems that cause the poisoning in people are thyroid tachycardia, palpitations, and tremors or increase in the sanguineous pressure, diseases, allergies, and to bring about the death by cardiac fault [14, 29, 35, 36].

USE STANDARDIZATION

The β-agonists authorized in Mexico and the United States as additives for cattle use and of use in bovines of get fat, are the hydrochlorate of zilpaterol (Zilmax) and the hydrochlorate of ractopamina (Optaflexx). Both have the characteristic of fast elimination and lowest residuality when these are suspended; these compounds are of the group of the fenetanolamins (β-agonists), unlike clenbuterol (CCL), that have an activity prolonged and therefore of high residuality with much more slow elimination than the previous ones, representing high toxicity to use it like additive [24].

On the matter of the NOM-061-ZOO-1999 [33], in the section of considerations, it indicates: "That the adoption of Good Practices of Feeding of the animal in the cattle operations diminishes the animal health risks and increases the productivity of the species destined to the human consumption."

"That due to the animal health risks and of public health that the use of some active ingredients represents as it is the case of clenbuterol in nutritional products destined for animal consumption, is indispensable to apply measures of restriction of immediate way."

And in Chapter 4, "Specifications," in its interjection 4,11 it mentions: It is prohibited the use of the following active ingredients and/or nutritional additives in the nutritional product formulation destined for consumption by animal:

- Violet crystal-like fungicide in raw materials and finished product.
- Cloranfenicol in its preventive or therapeutic modality.
- Coumarin in artificial fleibor´s.
- Pigment synthetic of the group of the Sudanese.
- Clenbuterol; as well as of all those nutritional ingredients and/or additives that can be injurious for the public health or represent animal health risk, and that do not count on the corresponding technical support for their use in the nutrition of the animal.

"The nutritional products finished, balanced foods, milky milk substitutes, supplements and all those foods that they include in his formulation, antimicrobials including in the Official Norm Mexican NOM-040-ZOO-1995 [32], whose indication is like therapeutic or modulator of the growth in the animal, must indicate in their label the legend 'food medicated.'

Of equal way in year 2002, the NOM-EM-015-ZOO-2002 [31], was elaborated. "Engineering specifications for the control of the use of β-agonists in the animal" in their fourth chapter "General Dispositions" mentions: "Except those products Beta-agonists that count on the registry and authorization of Secretaries (SAGARPA), for their use or consumption by animal, it is prohibited the production, manufacture, elaboration, preparation, preparation, transportation, traffic, commercialization, import, provision and/or use of the following active principles, additives, foods and/or medicines in nutritional product formulation destined for consumption and animal use, such as: Bromuterol, Carbuterol, Cimaterol, Cimbuterol, Clenbuterol, Fenoterol, Isoproterenol, Mabuterol, Orciprenalina, Pirbuterol, Ractopamina, Salbutamol, Terbutalina and Zilpaterol."

"The previous list has more no limiting a declarative purpose and includes any well-known beta-agonist or of new creation." "With the purpose to permanently watch and to supervise the dispositions anticipated in this Norm, the participants in this cattle activity, they must grant the necessary facilities to the Verifying Doctor, to realize properly its functions."

"The physical people or morals that participate in the commercialization and introduction of the cattle, their products and by-products will be responsible shared in common for it gets fat." "The Secretariat will promote the consumption of meat and products of origin animal, originating of cattle operations that count on the certification of free of residues of beta-agonists." In Chapter 5, "Laboratories," it mentions: "For the establishment of the presence or absence of residues of beta-agonists in animal, their products and by-products, as well as in products nutritional chemistry-pharmacists and for their consumption and animal use by these, the samples must be processed in the official laboratory and/or designated by the Secretariat for the observance of this Norm."

Federal law of Health animal of year 2007, mentions in its Title sixth, "*Of the Product Control for Use or Consumption Animal, Establishments and Activities and Services,*" in its Chapter I "Of the Product Control for Use or Consumption Animal": in their articles 91, 92 and 95 mention the following thing: Article 91. - La Secretaries (SAGARPA) will be authorized to determine, to evaluate, to consider, to register, to authorize or to certify: The maximum limits of allowed antibiotic residues, hormonal, chemical, toxic compounds and other equivalents, in origin goods animal destined for human consumption, as well as the time of retirement of these alive animal substances.

Mixed committee FAO/OMS of Nourishing Additive Experts recommended the following MRL (maximal residue limit by its abbreviations in English) of Clenbuterol for the bovines: 0.2 µg/kg in muscle and the fat, 0,6 µg/kg in the liver and the kidneys and 0,05 µg/litro of milk. From these values of MRL, the maximum daily ingestion would be 0,235 µg, on a base of daily food consumption of 300 muscle g, 100 liver g, 50 g of kidney and fat and 1,51 of milk [50].

It is required to reconstruct the norms to detail and to respect the time of retirement and pursuits would be due to realize in sign of levels of residues in end items and before the sacrifice in the hair of the animal. At the moment, it is alarming that it does not regulate the sanctions to the transgressors of the law. Severe penal sanctions to which they transgress the

law and they use the CCL or, for the case must settle down and be orchestrated, any prohibited medicine to foment the cattle production [50].

REPERCUSSIONS IN PUBLIC HEALTH

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Public health is defined as the set of tending preventive actions to conserve, to control, to watch and to improve the desirable conditions of health for all the population, causing in the individual the attitudes, values and the conduct adapted to motivate its participation to the benefit of the individual and collective health [57]. The nourishing security is a very important topic of public health in the developed countries. Without considering that the medicines that are administered to the animal possibly can be ingested by the humans, it even can be very small proportions in the form of residues. This exhibition to substances through foods is at the moment one of the most important subjects to the consumers and the cause of preoccupation in several countries, European and international organizations, due to the consequences that it can have in the health of the population [3].

The CCL is a adrenergic agonist β2 approved for their use limited in veterinary medicine in the United States. Their properties are similar to those of salbutamol and used sometimes in Europe like bronchodilator in humans. The lifter of weights uses clenbuterol of illicit way by their anabolic and lipoltics properties to increase their muscular mass. This product is used in the production of bovine meat to increase its muscular mass. Nevertheless, its use has been prohibited since the people who have consumed this meat have been intoxicated with CCL [6].

The foods with a high fat content are considered one of the main causes of cardiovascular diseases; thus, the consumers in the developed countries avoid consuming these foods. The cattle dealers use anabolic substances, like the β-agonists, since these mean that the meat has little fat content with high protein content—making this meat attractive to the consumer [37].

The competition in the intensive production of meat, the demand by greater production, preferably to the smaller cost and in the smaller possible time, with changes in the production of the cattle, are constant changes in the feeding of the cattle, improvements of the genetics of the animal and the control of the diseases between these [37].

This pressure is the one that has oriented to the illegal use of pharmacological agents, specifically anabolic compounds that promote the increase (EUA, directive no. 2003/74, 2003). The CCL, and β-agonist, is an authorized drug of veterinary use with therapeutic applications in bovines and horses among others animal species like bronchodilator or tocologic agent for cows. The use of these compounds as promotional of growth represents a risk for the public health, since cases of poisonings in several countries have appeared [3, 21].

The adrenergic agonists β2 are including in the list of substances that are prohibited in zootechnics in EUA (Directive no. 96/22/CE, 1996), and the CCL (4-amino [tert. - butilamin) metil] -3, 5-diclorobenzil alcohol hydrochlorate), is one of which more commonly they are used, since it increases the muscular mass and at the same time it promotes a decrement in the accumulation of the fat [3].

Nevertheless, although its use is prohibited, several cases of poisoning because of the liver ingestion and meat have been reported that contained residues of clenbuterol in Spain [8, 23], France [35, 36], Italy [4] and China [44].

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The government of Mexico, from 1999, emitted the Official Norm Mexican NOM-061-ZOO-1999 [33], which prohibited its use in the country. Despite their prohibition, cases of poisoning have appeared. The Federal Commission for the Protection against Sanitary Risks of the Secretariat of Health informed 136 affected people who consumed meat and vísceras of animals fed with CCL. The cases were concentrated in seven states of the country and D.F., mainly.

In the work of Zaragoza and Pedraza (2005) [56], when determining residues hydrochloride of clenbuterol in blood serum, of 582 samples, 153 were positive, representing a 26.29% of the total of collected samples.

It is important to emphasize that all the sectors involved in the production and supervision of the food processing have the obligation to try assure at the origin of the product that production animal is of high quality, which includes the monitoring of a level of residues of drugs agreed to the standards established by international organisms (Codex alimentarius, FDA, European Community, FAO/OMS among others); in this sense, the CCL is a medicine that gives to evil use by the interaction between its promotional effectiveness of the productive yield and its slow speed of purification, which without a doubt requires attention by the risk in public health [16, 53].

According to Elliot et al. [7], on the basis of the criterion of zero residues, a time of retirement of four weeks is recommended when a conventional dose is used to improve the yield of the channel; nevertheless, these authors do not make reference to the required time using other dosages of clenbuterol.

To promotional doses of the production or superiors, and mainly without a retirement in time and it forms of CCL, established 30-60 days, the problem of the illegal use of clenbuterol concentrates mainly in the risks that the product ingestion represents for the consumer of contaminated origin animal with this substance [19, 21].

In study realized by Valladares et al. [51], using the model murino determined the residual effect of the CCL and histological alterations in liver, considering the important paper of this weave in each organism, when receiving dose of 10, 15 and 20 mg/kg during a month, provided the product that is in the market; raising that by the particularities of the substance and their chlorine radicals, this tends to affect in significant way to the hepatocities, being caused initially congestion, hemorrhage, swelling and hydrospace degeneration, and predisposing to the secondary affection of "pathogens" by the infiltration of polimorfonucleares found.

And in studies realized as much in alive as test-tube investigators of the FAO-OMS, they considered that the CCL administration increased the pull-ovarian incidence of Leiomiomas after having received dose of up to 25 mg/kg of weight to the day; concluding that these must more to a adrenergic stimulation not as much and to factors of genotoxyc type. On the other hand, in rabbits in which they were administered doses of 30µg - 50 mg/kg/día, there have been observed signs of fetotoxyc like delay of ossification and cleaved palate [2]. It would be interesting to realize investigations of this type in bovines, birds or pigs, since these species are those that are consumed in major in amount and frequency in the national market [21].

In all Mexico, in illegal and clandestine way the distribution, commercialization and use of CCL takes place; nevertheless, the work with the cattle organizations for the registry of free units of CCL production will guarantee the consumption of meat; also proposing other substances of which until the moment they do not have toxicity indications - as the case of the

ractopamina and zilpaterol - will cause a viable productivity, safe and innocuous in the different units from cattle production [31, 33].

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Chapter 14

ROLE OF FAT ADDITION IN SHEEP REPRODUCTION

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ABSTRACT

The main objective of this chapter is to have a greater understanding of the role of adding fat to the diet in different presentations such as protected fat or seeds of some plants that have large amounts of fatty acids n-3 family and n-6, as well as some ingredients like fish meal and the effect of the fat in some reproductive variables. Those are necessary for proper reproductive performance in ruminants such as the secretion of luteinizing hormone (LH), Progesterone, Follicular Dynamics and Prostaglandins, showing that an alteration of these can influence the success or failure of the fertilization process and as a result of future pregnancy in the sheep or cow. It is expected that this chapter will wake up the interest of the reader in the composition of the fats and their role in reproductive performance.

INTRODUCTION

The extreme effects of nutrition in reproduction are widely recognized, and may have a negative effect in the reproductive performance of animals. However, animals have developed physiological mechanisms which allowed them to make decisions about the environment that surrounds them, achieving reproduction in the best conditions for their offspring to be born, for example, when food is abundant [1].

During the 1960´s, there was a great breakthrough on the regulation of the hypothalamic –hipofisis- gonad with the appearance of the determination on hormones concentration technique named radioimnmuno analyses. Later on, there have been other techniques such as

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ecography and DNA amplification, which allowed the enrichment of our understanding of what goes on in the ovary such as the follicular growth waves and the expression of receptors y different tissues, which intervenes in reproduction [2,3]

Even though there have been major advances in the reproduction area, it is still difficult to understand completely the relation between nutrition and reproduction. However, it has been proposed that the influence is straight over the ovary through nutrients such as glucose in its different categories or other fatty acids, achieving the elevation of glucose transporters in its different categories. On the other hand, it has also been proposed that there are other existing organs which mediate the effect of these nutrients, such as the liver, fat tissue and pancreas. These organs produce hormones that communicate to the brain about the current energetic state of the organism. However, the target zones of the brain or whether if there is an associated effect to other hormones, is not clear yet [4]

Reproduction in ruminants, is associated directly to the availability of energy and fat, both being important energy sources, for what it is suggested to favor the reproductive function, however, it has been suggested that the effects of fat in the diet may be independent from the contribution of the energy density on the diet and that the specific components of the fatty acids may stimulate ovary function, as suggested in the study done by Petit *et al.* [5] whom mentioned that fatty acids might influence fertility by acting as precursors for prostaglandins or also, how they might affect esteroidogenesis through the increase of cholesterol availability.

Recently , there has been a greater interest oin the eicosapenthanoic and docosahexanoic fatty acids, due to their apparent ability to reduce the uterine secretion of $PGF_{2\alpha}$ in the endometrial cells through the displacement of the arachidonic acid, precursor of the biosynthesis of $PGF_{2\alpha}$, both in vitro and in vivo [6].

Childs *et al.* [7] have reported evidence of an improvement on reproductive function after supplementing cattle with fish oil, which could be, in part, mediated through the systemic increase of cholesterol or well by an appropriate functioning of the luteum corpus, which will also cause an increase oin the concentrations of progesterone. It is also mentioned aAn elevation ofn the systemic concentrations of IGF-I is also mentioned, suggesting this as a consequence ofto the addition of fish oil which has an important role in increasing fertility [7]. Fatty acids in the cell might have a direct effect on encodinge protein gene´s transcription which are essential for reproductive events.

GENERALITIES

Fats are biological compounds soluble in organic solvents which include cholesterol, thriacilglicerides and phospholipids. The latterse last ones are part of the cellular membrane and play a role as a reserve of supply for fatty acids, necessary to synthesize a variety of efector molecules such as eicosanoids, a group of compounds that include prostaglandins, tromboxans and leucotriens. Mean while, cholesterol is also a compound of the cellular membrane and its function is to be a precursor in the synthesis of steroid origin hormones [6].

Fatty acids are an integral compound of the phospholipids bilayer of the cellular membrane which intervenes on the flow of the membranes through interactions between lipids and proteins. Therefore, fatty acids may affect protein and cellular's receptor

transportation for hormones and neurotransmisors. Also, fatty acids have the ability to influence on the expression of gene codification for enzymes, which are involved in lipid´s metabolism and interact with nuclear receptor proteins that bond with DNA. Therefore, fatty acids may alter regulatory gene´s transcription [8].

Fatty acids are composed ofby a hydrocarbon chain with a carboxyl terminal. The properties of fat on a diet are firstly determined by the composition of their fatty acids, which could be saturated (absence of a double union), mono saturated (presence of a double union) or poly saturated (more than one double union). Fatty acids may adopt two different geometric configurations, denoted as Cis or Trans configurations. Double- union Cis have two hydrogen atoms on the same side of the molecule, while Ttrans configurations have hydrogen atoms on the opposite sides of the molecule. Geometric isomerism has implications for shape and physics´s properties of the molecule, in which Cis' acids have a twisted chain and Ttrans fatty acids adopt a straight configuration and function as saturated fatty acids [9].

The structure of fatty acids is found based on the length of the acyl chain, the number of double unions that the chain and the types of isomers formed by each double union determines their functions, meanwhile saturated fatty acids don´t show double or triple unions on their acyl chain [9].

Unsaturated fatty acids are classified in different families based on the position found of their first double union in relation to the final methyl. Unsaturated fatty acids are classified in different families, based on the position where the first double bond is placed in relation with the final methyl. As an example, we have linoleic acid, which has 18 carbon atoms, with two double bonds represented with the next formula (c18:2), having the first double bond on the sixth position in relation with the final methyl, for which it is a member of the n-6 family. In contrast with linoleic acid, linolenic acid has 18 carbons on its acil chain, with a difference inon the number of double bonds, which are three, as shown in the next formula (c18:3), whenand the carbon number in which the first of this double bond presents itself which is on carbon number three of the cail chain, considering this type of chain, a fatty acid from the n-3 family [9,10]. The process of fatty acids will only generate fatty acids from that same family, for example, a fatty acid from the n-3 family canno´t become a member offrom the n-6 family and vice versa [9,10].

Fatty acids in vertebrates may be supplied through diet or be synthesized by themselves from other fatty acids, having an increase inon the number of carbons (elongation) through the action of the elongate enzyme, which adds two carbons. They can also suffer a process of desaturation which is catalyzed by desaturasas enzymes, adding a double union on the carbon chain. This kind of enzyme is classified according to the position of the double union's insertion, which, in this case, is between carbon 6 and 7seventh of the final carboxyl chain [9].

In domestic animals, desaturation of fatty acids occurs in positions higher than the 9th carbon position, to avoid synthesis of fatty acids from the family n-3 and n-6, in which dependence exists by theseis types of fatty acids. This takes into consideration to add these fatty acids to the diet. An example of this could be linoleic acid, (c18:2, n6) which is required for the synthesis of arachidonic acid (20:4, n6) and eicosanoids [11].

As fat supplies passes through the rumen, they suffer a series of modifications by the existing bacteria in the microbiota of ruminal flora, which digest thriacilglicerides, phospholipids and galactolipids liberating fatty acids off their glycerol esqueleton, once liberated, unsaturated fatty acids suffer a reduction oin its number of double unions. There is

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also a change in the orientation of the isomers called biohydrogenationhidrgenation process, however, some polyunsaturated fatty acids of the family n-3, such as eicosapenthanoic acid (20:5, n-3) and docosahexaenoic acid (22:6, n-3) have showned to experience a biohydrogenationhidrogenation [12]. Eicosapenthaenoic and docohexanoic acids are found typically in ingredients derived from fish such as oil and flour. The fat supplement, partially resistant to biohyidrogenation, such as long- chain fatty acids calcium salts (CalCFA), have been developed to increase the amount of unsaturated fatty acids that reach the duodena to be incorporated to fat tissue and milk [10].

Fatty acids calcium salts were originally developed at the early 1980's at the University of Ohio in thelike a form of fat in the rumen to avoid ruminal fermentation and digestive problems. In the early 1990´s, they received attention for partially escaping biohiydrogenation [8]. For example, Wu *et al.* [14], reported a 49 % of biohyidrogenation in diets containing calcium salts with palm oil compared to an 80% of biohyidrogenation in diets containing animal-vegetal fat; Klusmeyer and Clark [16], equally found less biohyidrogenation in diets supplemented with calcium salts when compared with the control diet.

DIETARY FAT EFFECT ON LH SECRETION

It has been observed that a negative energetic stage reduces the frequency of LH pulses, which are necessary for the ovary follicles to grow during the pre-ovulatory stage in cattle and sheep [16]. Frequently, milk cattle areis supplemented with fat in feeding rations to increase the energy concentration in diets, which results in an increase on the energy consumption and in an improvement of the energetic state of the cow. Harrison *et al.* [17], observed that the energy provided by energy supplementation increased LH secretion in animals compared to those who consumed less energy than required [18,19]. Even though there is mechanism energy independent from which fatty acids affect LH secretion, it has not been established in ruminants [20].

DYNAMIC FOLLICULAR

Some studies have shown that complementation in feeding with fat, alters the growth dynamics of the follicle and the number of follicles recluted from the ovary, and that this effect is energy independent. Oldick *et al.* [21] observed that that feeding cattle with fat stimulated follicle's growth during the pre-ovulatory stage, also in other studies similar to this one, the total number of follicles increased [22,23], taking into consideration that this response may be due to an increase in LH concentrations in plasma, stimulating the last stages of follicular growth. On another study by El-shahat and Abo-el Maaty [24], in Rhmani sheep, it was confirmed what was previously explained in cattle, mentioning that the use of long-chain fatty acids calcium salts, increased the number of medium and large follicles, destined to ovulate.

However, Wonnacott *et al.* [25], reported that feeding sheep with diets rich inwith fatty acids from de n-3 or n-6 families, were incapable of altering the size or number of follicles as

an effect of the diet., mMore research is necessary to determine which types of fatty acids are needed to alter the amount of follicles and their size.

FUNCTION OF LUTEUM CORPUS AND STERIOGENIC

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It has been proved in cows that fat supplementation increases the total concentration of cholesterol in the follicular fluid, in relation to the granulose cells from cow´s follicles fed with fat showed an increase oin progesterone and androstenedione *in vitro* [26]. The previous explanation agrees with the reported by Wonnacott *et al.* [25], whom observed that sheep fed with diets rich inwith unsaturated fatty acids from the n-3 family by a period of 6 weeks, had higher concentrations of progesterone in the follicular fluid in comparison with sheep fed with diets rich inwith polyunsaturated fatty acids from thede n-6 family.

As previously described, fat addition in diets may increase follicular size in the last stages of the development and develop a largerbigger sized luteum corpus increasing its steriogenic ability, reflecting on little increases on the concentrations of progesterone in blood plasma [27]. It, also, suggests that fat may increase the concentrations of cholesterol in blood plasma [28], which is necessary for the synthesis of progesterone, indicating that a major availability of cholesterol results in an increase of progesterone´s secretion. However, it has been noted that incubation of cows' lutheal cells with PUFA's such as eicosapenthanoic and docohexanoci acids, decrease the secretion of progesterone [29].

It was seen that a daily intravenous infusion of long chain fatty acids (soy oil) in sheep during the half or at the end of diestrus has led to an increase in cholesterol and progesterone concentrations in blood plasma [30]. On the other hand, Espinoza *et al.* [31], mentions that pelibuey sheep showed a higher cholesterol concentration when fed with overweight fat and observed the same tendency in progesterone´s concentration on the 12^{th} day of the estrus cycle. This was confirmed by El-shahat y Abo-el Maaty [19], who fed Rhmani sheep diets with long- chain fatty acids calcium salts during a estrus synchronization, program reporting an increase in blood plasma progesterone concentrations. Nevertheless, Espinoza *et al.* [31,32] mentioned that the addition of fat protected at a 1.5 % of the diet or cow fat at a 1.2%, did not affect progesterone concentrations in a period of 75 days. The increase of progesterone concentrations in blood plasma on the lutheal fase before and after insemination has been associated with high pregnancy rates [33], suggesting that progesterone plays an important part in the preparation of the uterus for the embryo implantation.

In cows, there is evidence that the removal rate of progesterone in blood plasma is decreased when animals are supplemented with fats such as those shown by Hawkins *et al.* [34]. They removed the luteum corpus in cows and observed that the decrease rate of progesterone´s concentration was less in cows fed with a supplement of CalCFA compared to the ones not fed with this supplement. This effect on the removal rate of progesterone in blood plasma was, also, provend by Galbreath *et al.*[35] with ovariectomized sheep, in which a CIDR was implanted with natural progesterone. However, they only evaluated two fatty acids sources integrated in diets such as linseed and flax´s seed, and reported that sheep fed with linseed, showed a higher progesterone´s absorption and the disappearance rate of progesterone in blood serum was faster in comparison with the results in sheep fed with flax´s seed [35].

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PROSTAGLANDINS

Prostaglandins are bioactive compounds derived ofby fatty acids of 20 carbons such as arachidonic acid. It could be acquired through diet or synthesized by the organism itself from linoleic acid, involving the desaturation on the positions 5 and 6 and an elongation of 2 carbons catalyzed by the enzyme elongaze such as in the case of series 2 prostaglandins ($PGF_{2\alpha}$), which are important regulators of labor and in some species, they cause regression of the corpus luteum which will guide the start of a new estral cycle [9,10].

Arachidonic acid is kept as phospholipids in the membrane, having high concentrations in uterine rat tissue. For the formation of series 2 prostaglandins, it has to be liberated from the membrane through the action of the a2 phospholipase. Once it is out of the membrane, this can be processed by the PGHs enzyme complex in order to produce prostaglandins and tromboxans [10].

Burke *et al*. [30], reported that the intravenous infusion of long- chain fatty acids in a daily dose of 250 ml of soy oil, increased the concentrations of $PGF_{2\alpha}$ and PGE_2 in ovariectomized sheep, Cheng *et al.* [36], obtained in uteri epithelial cells from synchronized sheep during reproductive season, and found that the addition of gammaminoleic acid produced changes in the response from the epithelial uterine cells to oxitoxine, increasing the production of $PGF_{1\alpha}$, which was significantly elevated. Supplementation with arachidonic acid augmentated the production of the three isomers ($PGF_{1\alpha,}$ $PGF_{2\alpha,}$ $PGF_{3\alpha,}$), $PGF_{2\alpha,}$ in particular.

Changes oin progesterone's profile during the estral cycle could probably affect the beginning of the embrionary development indirectly. It has been noticed that the decrease on the concentration of progesterone in the beginning of the luteum phase in cattle, reduces the embryos survival, an effect thought to be mediated through changes in the uterine secretions [37]. On the other hand, the ability of the supplements with PUFA's n-3 to delay luteolisis has been proposed as a potential method of improvement on cattle´s fertility for giving the product a major development before thede beginning of luteolisis [38].

On the 15th day, Mattos *et al.* [39] reported a decreased secretion of the $PGF_{2\alpha}$ metabolite in cows fed with fish flour, but Petit *et al.* [40] found a high response in cows fed with a mixture of linseed and fish oil compared with the ones that were only fed with linseed.

Petit *et al.* [5] reported a higher tendency to the production of PGFm on the 15th day on a diet supplemented with sun flower seed compared to linseed or megalac. However, Robinson *et al.* [41] did not found effects of linoleic or linolenic on diets based on it during the days 15th and 16th days of the estral cycle.

These results suggest that changes oin the diet involving PUFA's might influence the response to the $PGF_{2\alpha}$ metabolite, however, it hasn't emerged as a constant pattern. This can be due to the fact that quantities of PGFm liberated in response to the increase of oxitocine also elevates with the approximation to lutheolisis. Therefore, the differences in the treatments might be confused with light changes in the length of the cycle between the animals [40].

There is evidence that linoleic eicosapenthanoic acid, inhibit the liberation of $PGF_{2\alpha}$ in the endometrial cells in cows. Meanwhile, in cycling sheep, it has been shown that a diet with high linoleic acid delays the luteus regression through the reduction of the PGF metabolite 13,14-dihifro-15-keto- $PGF_{2\alpha}$ (PGFm) [39]. Petit *et al.* [40] reported a higher concentration of

this PGFm in cows that received a mixture of linseed and fish oil compared to those that receivedgot linseed alone. Also, Petit *et al*. [5], reported a major tendency to a higher concentration of PGFm on the 15th day with a supplemented diet with sun flower seed compared to flax seed or overweight fat. However, Robinson *et al*. [38] did not findound any effect on the concentrations of PGFm in diets based on linoleic acid ion the days 15th, 16th andy 17th days of the cycle. The previous information suggests that changes in the diet involving PUFA's can influence the response of PGFm, but there has not been seen a consistent pattern to it has not been seen [6].

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GESTATION

In experiments where cow´s diets were with fish flour, which is rich with fatty acids from the n-3 family, conception rates were improved. However, results frequently are variable and are found far from being conclusive, such as results reported by Ambrose *et al.* [42]. Petit and Twagiramungu [43], who noted that including flax seed (rich with n-3 fatty acids), in the diet decreased fertility rates in meat cattle. There was no increase in the fertility by diets with fatty acids calcium salts in sheep. Nieto *et al.* [44], however, suggested that fatty acids from the n-3 or the n-6 family may influence the composition and development of the oocytes and embryos [25].

CONCLUSION

Fats play an important role in follicular dynamics and not only for the energy density but the type of fatty acids that conform them., aAbove statements, reveals that fatty acids may influence follicular development and future function of the corpus luteum, which, depending on follicular development, will be able to generate adequate amounts of progesterone that allows the success of future pregnancy, however, it is interesting that some fatty acids have the ability such as eicopentanoico have the ability to inhibit the production of PGF2α., however, sSunflower seeds, however, in its composition of fatty acids, can increase the production of PGF2α, although there is a breakthrough in understanding the role of different fatty acids contradiction still exists in some studies with respect to the role they play in the reproductive capacity of ruminants.

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