In: Nutritional Strategies of Animal Feed Additives
Editor: Abdel Fattah Z. M. Salem
ISBN: 978-1-62417-000-3

Chapter 1

ANAEROBIC ENZYMES AS A NEW TECHNOLOGY IN ANIMAL FEED

H. Gado[1] and A. Z. M. Salem[2]*
[1]Ain Shams University, Department of Animal Production, faculty of Agriculture, Cairo, Egypt
[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Estado de México, México

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

* Corresponding author: asalem70@yahoo.com.

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].

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]

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.

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].

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].

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, 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].

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

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.

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.

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.

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.

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.

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 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.

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.

REFERENCES

[1] Falcón, M. A., Rodríguez, A., Carnicero, A., Regalado, V., Perestelo, F., Milstein, O., and De La Fuente, G. (1995). Isolation of microorganisms with lignin transformation potential from soil of Tenerife Island. *Soil Biol. Biochem.* 27(2), 121-126.

[2] Weimer, P. l. (1996). Why don't ruminal bacteria digest cellulose faster? *J. Dairy Sci.* 79, 1496-1502.

[3] Forsberg, C. W. and Latin, K. (1977). Use of adenosine 5-triphosphate as an indicator of the microbiota biomass in rumen contents. *Appl. Environ. Microbiol.* 33, 528.

[4] Van Soest, P. J. (1982). Nutritional ecology of the ruminants. B Books. Inc., Oregon, US.

[5] McAllister, T. A., Hristov, A. N., Beauchemin, K. A., Rode, L. M., and Cheng, K.-J. (2001). Enzymes in ruminant diets. In: Bedford, M., Partridge, G. (Eds.). *Enzymes in Farm Animal Nutrition*. CABI Publishing, Oxon, UK, pp. 273–298.

[6] Phipps, R. H., Sutton, J. D. and Bhat, M. K. (2002). Are enzymes useful in ruminant diets? *Proce. of the BSAS Annual Meeting,* York, England, 8-10 April. pp. 249-250.

[7] Nussio, L. G., Huber J. T., Theurer, C. B., Nussio, C. B., Santos, J., Tarazon, M., Lima-Filho, R. O., Riggs, B., Lamorreaux, M., and Treacher, R. J. (1997). Influence of a cellulose/xylanase complex (C/X) on lactational performance of dairy cows fed alfalfa hay based diets. *J. Dairy Sci*. 80 (Suppl. 1), 220.

[8] McAllister, T. A., Stanford, K., Bae, H. D., Treacher, R. J., Hristov, A. N., Baah, J., Shelford, J. A., and Cheng, K. J. (2000). Effect of a surfactant and exogenous enzymes on digestibility of feed and on growth performance and carcass traits of lambs. *Can.J. Anim. Sci.* 80, 35–44.

[9] Svozil, B. J., Votava, P., Zobac, and Horak, V. (1989). Application of the cellulolytic preparation in nutrition of lambs. *Sborik Vedeckych Praci Vyzkummy Tsta Vyzivy Zvirat Pohorelice*. 22, 69-78.

[10] Beauchemin, K. A., Yang, W. Z., Morgavi, D. P., Ghorbani, G. R., Kautz, W., and Leedle, J. A. Z. (2003). Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. *J. Anim. Sci*. 81, 1628–1640.

[11] Nsereko, V. L., Morgavi, D. P., Rode, L. M., Beauchemin, K. A., and McAllister, T. A. (2000). Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms *in vitro*. *Anim. Feed Sci. Technol*. 88, 153–170.

[12] Gado, H. M. and Salem, A. Z. M. (2008). Influence of exogenous enzymes from anaerobic source on growth performance, digestibility, ruminal fermentation and blood metabolites in lambs fed orange pulp silage in total mixed ration. *In: 59th Annual meeting of the European Association for Animal Production, 24-27 August,* pp. 228, Vilnius, Lithuania. Poster.

[13] Salem, A. Z. M., Hassan, A. A., Kalil, M. S., Yacout, M. H., and Gado, H. (2007a). Feed intake, digestibility coefficients, nitrogen utilization and loss of secondary compounds in whole gastrointestinal tract in sheep feed Atriplexhalimusfoliages in presence of ZADO® as direct fed microbials. *The 12th Seminar of the FAO - CIHEAM Sub-Network on Sheep and Goat Nutrition: Nutritional and foraging ecology of sheep and goats Thessaloniki, Greece*.

[14] Gado, H. M., Metwally, H. M., Soliman, H., Basiony, A. Z. L., and ElGalil, E. R., (2007). Enzymatic treatments of bagasse by different sources of cellulase enzymes. *In: The 11th Conf. Animal Nutr.*, Al-Aqsor-Aswan, Egypt on 2 November, 13–18. 10, p. 607.

[15] Soliman, M. S. (2006). Utilization of peanut hay in ruminant feeding. *Ph.D. Thesis.* Alexandria University, Alexandria, Egypt.

[16] Gado, H. M., Salem, A. Z. M., Robinson, P. H., and Hassan, M. (2009). Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. *Anim. Feed Sci. Technol*.154 (1-2), 36-46.

[17] Khattab, H. M., Gado, H. M., Kholif, A. E., Mansour, A. M., and Kholif, A. M. (2011). The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. *Inter. J. Dairy Sci.* 6 (5), 267-277.

[18] Gado, M. and Borhami, B. E. (2011). Effect incremental levels of ZADO® on milk production and rumen parameters. *62th Annual Meeting of the EAAP*. Satavenger. Norway. pp 271.

[19] Gado, H., Hassan, M. and Salem, A. Z. M. (2008). Effect of ZADO®, as enzymes from anaerobic bacterium, on extent of ruminal fermentation kinetics, microbial protein synthesis and milk production in dairy cows. In: ADSA PSA AMPA ASAS Joint Annual Meeting, Indianapolis, July 7-11, Indiana, US. pp 590-591.

[20] Allam, S. M., El-Shaer, H. M., Yuossef, K. M., Ali, M. A., and Bakr, S. Y. A. (2009). Impact of feeding biologically treated wheat straw on the production performance of goats in North Sinai. *World Journal of Agricultural Sciences* 5(5), 535-543

[21] Rode, L. M., Yang, W. Z. and Beauchemin, K. A. (1999). Fibrolytic enzyme supplements for dairy cows in early lactation. *J. Dairy Sci*. 82, 2121–2126.

[22] Schingoethe, D. J., Stegeman, G. A. and Treacher, R. J. (1999). Response of lactating dairy cows to a cellulase and xylanase enzyme mixture applied to forages at the time of feeding. *J. Dairy Sci*. 82, 996–1003.

[23] Kung, L. Jr., Treacher, R. J., Nauman, G. A., Smagala, A. M., Endres, K. M., andCohen, M. A. (2000). The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. *J. Dairy Sci*. 83:115–122.

[24] Bowman, G. R., Beauchemin, K. A. and Shelford, J. A. (2002). The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. *J. Dairy Sci*. 85, 3420–3429.

[25] Dhiman, T. R., Zaman, M. S., Gimenez, R. R., Walters, J. L., and Treacher, R. (2002). Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding. *Anim. Feed Sci. Technol*. 101,115–125.

[26] Knowlton, K. F., McKinney, J. M. and Cobb, C. (2002). Effect of a direct-fed fibrolytic enzyme formulation on nutrient intake, partitioning, and excretion in early and late lactation Holstein cows. *J. Dairy Sci*. 85, 3328–3335.

[27] El-Ashry, M. A., Kholif, A. M., Fadel, M., El-Alamy, H. A., El- Sayed, H. M., and Kholif, S. M. (2003). Effect of biological treatments on chemical composition *in vitro* and *in vivo* nutrients digestibilities of poor quality roughages. *Egyptian J. Nutr.Feeds* 6 (2), 113 – 126.

[28] Sutton, J. D., Phipps, R. H., Beever, D. E., Humphries, D. J., Hartnell, G. F., Vicini, J. L., and Hard, D. L. (2003). Effect of method of application of a fibrolytic enzyme product on digestive processes and milk production in Holstein-Friesian cows. *J. Dairy Sci*. 86, 546–556.

[29] Titi, H. and Lubbadeh, W. F. (2004). Effect of feeding cellulase enzyme on productive responses of pregnant and lactating ewes and goats. *Small Rum.Res.* 52, 137–143.

[30] Gado, A. M., Gado, H. and Metwally, H. M. (2005). Effect of ZADO® as a biological additive on the performance of small ruminants fed rations based on agriculture by-products. *Res. Bult*. Ain ShamsUniv. 2005-1.

[31] Eun, J. S., Beauchemin, K. A., Hong, S. H., and Bauer, M. W. (2006). Exogenous enzymes added to untreated or ammoniated rice straw: Effects on *in vitro* fermentation characteristics and degradability. *Anim. Feed Sci. Technol.*131, 86–101.

[32] Kholif, S. M. (2006). Effect of improving the nutritional value of poor quality roughages on the yield and composition of goat's milk. *Egyptian J. Dairy Sci*. 34, 197-205.

[33] Tricarico, J. M., Abney, M. D., Galyean, M. L., Rivera, J. D., Hanson, K. C., McLeod, K. R., and Harmon, D. L. (2007). Effects of a dietary *Aspergillus oryzae* extract containing -amylase activity on performance and carcass characteristics of finishing beef cattle. *J. Anim. Sci*. 85, 802-811.

[34] Salem, A. Z. M., El-Adawy, M., Gado, H., and Khalil, M. (2007b). Feed intake, nutrient digestibility and animal growth performance in sheep and goats fed wheat straw ad lib. in presence of ZADO® as direct feed of anaerobic enzymes and bacteria. *J. Anim. Sci.* 85 (Suppl. 1). *Dairy Sci.* 90 (Suppl. 1). *Poult. Sci*. 86 (Suppl. 1).

[35] Lewis, G. E., Hunt, C. W., Sanchez, W. K., Treacher, R., Pritchard, G. T., and Feng, P. (1996). Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. *J. Anim. Sci*. 74, 3020–3028.

[36] Beauchemin, K. A., Yang, W. Z. and Rode, L. M. (1999). Effects of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. *J. Dairy Sci.* 82, 378–390.

[37] Yang, W. Z., Beauchemin, K. A. and Rode, L. M. (1999). Effects of an enzyme feed additive on the extent of digestion and milk production of lactating dairy cows. *J. Dairy Sci.* 82, 391–403.

[38] Bowman, G. R., Beauchemin, K. A. and Shelford, J. A. (2002). The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. *J. Dairy Sci*. 85, 3420–3429.

[39] Allam, S. M., Al- Bedawi, T. M., El-Amary, H. H., and Mohamed, S. H. (2006). Improving sugar beet pulp through biological treatment and its use in sheep ration. *Egyptian J. Nutr.Feeds* 9 (2), 235-247.

[40] Gado, H., Sohair, A. N., Mohamed, B. K., and Mahrous, A. A. (2006). Effect of biological treatments on the nutritive value of rice straw. *Egyptian J. Nutr.Feeds* 9 (2), 207-219.

[41] Borhami, B., Soliman, S., El-Adawy, M., Ghonaim, E., Yacout, M., and Gado, H. (2007). Biological treatment of peanut hay as ruminant feed. Joint annual meeting. San Antonio, TX. July 8-12.

[42] Bakshi, M. P. S. and Langar, P. N. (1985). Utilization of *Agaricus Bisporus* harvested spent wheat straw in buffaloes. *Indian J. Anim. Sci*. 55 (12), 1060-1063.

[43] Ahuja, A. K., Kakkar, V. K., Garcha, H. S., and Mokkar, G. S. (1986). Spent paddy straw as a basal roughage for sheep. *Ind. J. Anim. Sci.* 56, 285-287.

[44] Henics, Z. (1987). Wheat straw upgraded by *Pleurotus ostreatus*. *World Review of Animal Production*, XX 111, No.4, October- December.

[45] Deraz, T. A. (1996). The production of microbial protein from some agricultural wastes and its utilization in ruminant. Ph.D. Thesis, faculty of agriculture. Ain- Shams University.

[46] Kholif, S. M. (2001). Effect of biological treatment of low quality roughage on milk yield and composition. Ph.D. Thesis. Fac. Agric. Ain Shams Univ.

[47] Theurer, B., Woods, W. and Burroughs, W. (1963). Influence of enzyme supplements in lamb fattening rations. *J. Anim.Sci.* 22, 150–154.

[48] Gado, H. (1997). Effect of enzymatic treatments for poor quality roughages on fiber digestibility and nitrogen metabolism in Baladi goats. *Egyptian J. Nutr. Feeds* 50–56 (specialissue).

[49] Knowlton, K. F., McKinney, J. M. and Cobb, C. (2002). Effect of a direct-fed fibrolytic enzyme formulation on nutrient intake, partitioning, and excretion in early and late lactation Holstein cows. *J. Dairy Sci*. 85, 3328–3335.

[50] Zewil, M. G. (2005). Evaluation of some treatments for rice straw. Thesis master of science. *Anim. Prod. Deprt.*, Fac. Of Agric., Al azhar. University.

[51] Gado, H. M., Salem, A. Z. M., Odongo, N. E., and Borhami, B. E., (2011). Effect of exogenous enzymes ensiled with orange pulp on digestion, blood metabolites and growth performance in lambs. *Anim. Feed Sci. Technol*.165, 131–136.

[52] Hristov, A. N., Rode, L. M., Beauchemin, K. A., and Wuerfel, R. L. (1996). Effect of a commercial enzyme preparation on barley silage *in vitro* and *in sacco* dry matter degradability. *Proc. West. Sect. Am. Soc. Anim. Sci*. 47, 282–284.

[53] Bhat, M. K. and Hazlewood, G. P. (2001). Enzymology and other characteristics of cellulases and xylanases. *Page 11 in Enzymes in Farm Animal Nutrition.* M. Bedford and G. Partridge, ed. CABI Publishing, Oxon, U.K.

[54] Eun, J. S. and Beauchemin, K. A. (2007). Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using *in vitro* fermentation characteristics. *Anim. Feed Sci. Technol*.132, 298–315.

[55] Dado, R. G. and Allen, M. S. (1995). Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. *J. Dairy Sci*. 78, 118.

[56] Oba, M. and Allen, M. S. (2003). Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. *J.Dairy Sci.* 86 (1), 195–207.

[57] Zadrazil, F. and Brunnert, H. (1981). Investigation of physical parameters important for the solid state fermentation of straw by white-rots fungi. *European J. Appl. Microbiol. Biotechnol.* 11, 138-188.

[58] Langer, P. N., Sehagal, J. P., Rana, V. J., Singh, M. M., and Garcha, H. G. (1982). Utilization of *Agaricus bisporus* cultivated spent wheat straw in the ruminant diet. *Indian J. Anim. Sci*. 52 (8), 643-647.

[59] Pearce, G. R. (1984). Characteristics of cereal straws in relation to their digestion. In: Doyle, P. T. (ed), The utilization of fibrous agriculture residues in animal feeds. School of Agriculture and Forestry, University of Melbourne, Parkville, Victoria, Australia. p.53.

[60] Zainur, A., Sharif, A. and Fadzil, M. Z. (1984). The utilization of rice straw stored for different time periods by sheep. In: Doyle, P. T. (ed), The utilization of fibrous agricultural residues in animal feeds. School of Agriculture and Forestry, University of Melbourne, Parkville, Victoria, Australia. pp. 72 – 73.

[61] Adesogan, A. T. (2005). Improving forage quality and animal performance with fibrolytic enzymes. In: *Florida Rum. Nutr. Symp.*, pp. 91–109.

[62] Jaster, E. H. and Moore, K. I. (1988). Fermentation characteristics and feeding value of enzyme-treated alfalfa haylage. *J. Dairy Sci*. 71, 705.

[63] Kung, L. J. R., Carmean, B. R. and Tung, R. S. (1990). Microbial inoculation or cellulase enzyme treated of barley and vech silage harvested at three maturities. *J. Dairy Sci.* 73, 1304-1311.

[64] Stokes, M. R. (1992). Effects of an enzyme mixture, an inoculant, and their interaction on silage fermentation and dairy production. *J. Dairy Sci*. 75, 764–773.

[65] Fredeen, A. H and McQueen, R. E. (1993). Effects of enzyme additives on quality of alfalfa/grass silage and dairy cow performance. *Can. J. Anim. Sci*. 73:581.

[66] Chen. K. H., Huber, J. T., Theurer, C. B., Swhgle, R. S., Simas, J., Chan, S. C., Wu, Z., and Sullivan, J. L. (1994). Effect of steam flaking of corn and sorghum grains on performance of lactating cows. *J. Dairy Sci*. 77:1038.

[67] Yang, W. Z., Beauchemin, K. A. and Vedres, D. D., (2002). Effects of pH and fibrolytic enzymes on digestibility, bacterial protein synthesis, and fermentation in continuous culture. *Anim. Feed Sci. Technol*. 102, 137–15.

[68] Dean, D. B., Adesogan, A. T., Krueger, N., and Littell, R. C. (2005). Effect of fibrolytic enzymes on the fermentation characteristics, aerobic stability, and digestibility of Bermudagrass silage. *J. Dairy Sci*. 88, 994–1003.

[69] Giraldo, L. A., Tejido, M. L., Ranilla, M. J., and Carro, M. D. (2007). Effects of exogenous cellulase supplementation on microbial growth and ruminal fermentation of a high-forage diet in Rusitec fermenters. *J. Anim. Sci*. 85, 1962-1970.

[70] Meng, Q. X., Xiao, X. J., Yu, H., Zhou, W. S., and Qiao, Z. G. (1999). Treatment of wheat straw with microorganisms and the nutritive value of treated straw as feeds for growing beef cattle. *Chinese J. Anim. Sci.* 35(6), 3-5.

[71] Zhang, Y. and Meng, D. L. (1995). Use of microbial ensiled wheat straw to feed dairy cattle. *China Dairy Cattle*. 6, 18-19.

[72] Xiao, X. J. (1998). Chemical composition and *in vitro* digestibility of wheat straw before and after treatment with white rot fungi. Undergraduate student thesis, China Agricultural University.

[73] Mahrous, A. A. and Faten, F., Abou Ammou (2005). Effect of biological treatments for rice straw on the productive performance of sheep. *Egyptian J. Nutr. Feeds* 8(1) *Special issue*, 529 – 540.

[74] Ho, Y. W., Abdullah, N. and Jalaludin, S. (1990). Invasion and colonization by anaerobic rumen fungi. In The Rumen Ecosystem. Proceedings of VII ISRP Satellite Symposium. Hakone, Japan. pp. 101–108 [S. Hoshino, R. Onodera, H. Minato and H. Itabashi, editors]. Berlin: Springer-Verlag.

[75] Perdok, H. B. and Leng, R. A. (1991). Effect of supplementation with protein meal on the growth of cattle given a basal diet of untreated or ammoniated rice straw. *Asian-Austral. J. Anim. Sci.* 3(4), 269–279.

[76] Silva, A. T. and Ørskov, E. R. (1988b). Fibre degradation in the rumens of animals receiving hay and untreated or ammonia treated straw. *Anim. Feed Sci. Technol*. 19, 277–287.

[77] Wang, A. (1998). Studies on dietary application of cellulose complex in monogastric and ruminant animals. *J. Northeast Agric. Univ*. 29(3), 236-251.

[78] Chen, X. B., Mathieson, J., Hovell, F. D., De, B., and Reeds, P. J. (1990). Measurement of purine derivatives in urine of ruminants using automated methods. *J. Sci. Food Agric*. 53, 23–33.

[79] Henderson, A. R. and McDonald, P. (1977). The effect of cellulose preparations on the chemical changes during the ensilage of grass in laboratory silos. *J. Sci. Food Agric*. 28, 486-490.

[80] Henderson, A. R., McDonald, P. and Anderson, D. (1982). The effect of a cellulase preparation derived from Trichadennaveride on the chemical changes during the ensilage of grass, lucerne and clover. *J. Sci. Food Agric*. 33:16.

[81] Stokes, M. R. and Chen, J. (1994). Effects of an enzyme-inoculant mixture on the fermentation of corn silage. *J. Dairy Sci*. 77, 3401-3409.

[82] Stokes, M. R. and Zheng, S. (1995). The use of carbohydrase enzymes as feed additives for early lactation cows. *23rd Biennial Conf. Rumen Function, Chicago, IL 23:35*.

[83] Hoffman, P. C., Welch, D. A. and Brehm, N. M. (1995). Potential of enzyme mixtures to improve silage quality and lactation performance of dairy cattle. *J. Prod. Agric.* 552-557.

[84] Sheperd, A. C. and Kung, K. L. (1996). An enzyme additive for corn silage: effects on silage composition and animal performance. *J. Dairy Sci*. 79, 1760–1766.

[85] Nadeau, E. M. G., Buxton, D. R., Russell, J. R., Allison, M. J., and Young, J. W. (2000a). Enzyme, bacterial inoculant and acid effects on silage composition of orchardgrass and alfalfa. *J. Dairy Sci*. 83, 1487-1502.

[86] Eun, J. S., Beauchemin, K. A. and Schulze, H. (2007). Use of exogenous fibrolytic enzymes to enhance *in vitro* fermentation of alfalfa hay and corn silage. *J. Dairy Sci*. 90, 1440-1451.

[87] Eun, J. S., Beauchemin, K. A. and Schulze, H. (2007). Use of an *in vitro* fermentation bioassay to evaluate improvements in degradation of alfalfa hay due to exogenous feed enzymes. *Anim. Feed Sci. Technol*. 135, 315–328.

[88] Kung, L. Jr. Schmidt, R. J., Ebling, T. E., and Hu, W. (2007). The Effect of Lactobacillus buchneri 40788 on the Fermentation and Aerobic Stability of Ground and Whole High-Moisture Corn. *J. Dairy Sci.* 90, 2309-2314.