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ROLE OF PROBIOTICS IN ANIMAL NUTRITION

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ABSTRACT

The anaerobic probiotic technology (ZAD) is a patented technique. The products are in two forms either liquid or powder. The powder products are coated so it can handle the pressure and the temperature of the extruders. They are fed as follows; 10 gm/head /day for large animals or 1 kg/ tone of fed (for ZADO). In case of Liquid (ZAD); it is fed 10 ml/head/day for large animals and 1.5 ml liter for poultry and rabbits. It decreases the aflatoxins less than 11% by inclusion rate of 7% of ZAD in 7 hours. They are helping the animals to cope with heat stress. Their ability to decrease the cholesterol and triglycerides are noticed by 21 and 19%, respectively. The birds and animal immunity is elevated too. Animal performances are improved in animals fed either form. Milk production increased in dairy cattle (1.5 kg/animal/day) and dairy buffaloes (1kg/head/day). The same trend was recorded for meat production in cattle, buffaloes, and sheep (220, 180 and 90 gm/head/day, respectively as an average daily gain). For poultry, it helped in decreasing the fattening period by 5 days. In fish, the final weight increased by 18%. For rabbits, it increased the milk production, decreased the mortality rate, increased the average daily gain, and improved all over physiological aspects of rabbits.

Contribution/Originality: This study documents the impact of probiotics on the growth performances and nutritional aspects of dairy animals, poultry, rabbit, fish, and beef. The primary contribution of this review article is to analyze the influence of anaerobic probiotic technology on the performances and other physiological aspects of ruminant and non-ruminant animals.

1. INTRODUCTION

Probiotics are live microorganisms that confer health benefits to the host, and are generally regarded as safe. The fungal source probiotics had been known for long time, but now it has been shifted towards the bacterial side, particularly the anaerobic ones. In fact, the anaerobic bacteria sources associated probiotics have better performance with animals. In terms of their nutrition, fungi are saprophytes, and are commonly found in soil or water constituting organic wastes. Digestive enzymes of fungi play a pivotal role in breaking down food outside their bodies, and help them absorb the degraded foods through cell walls, thereby considering them heterotrophic organisms. In contrary to this, bacteria belong to either heterotrophs or autotrophs.

Enzymes are known as biological catalysts that consist of diversified types of bioactive proteins that drive the chemical reaction by acting upon substrates through specific mode of action. Catalysis is the process in which enzymes produced by all living organisms trigger the chemical reactions. Substrates bind to the active site of the

enzyme and form products. According to lock and key model, each active site on the enzyme is unique for specific substrate. However, active sites have the tendency to change their shape in order to bind with substrate through the induced fit model, which moves entire protein domains. Several parameters viz. pH, temperature, substrate concentration, allosteric inhibitors and activators, and cofactors influence the activity of enzymes. An elevation in temperature (either below or above the optimum) causes the denaturation of active site, thereby avoiding the binding of substrate. On the other hand, at low temperature, enzymes lacks flexibility in order to allow induce fit. Substrate concentration is another crucial factor that affects the enzyme yield. If the concentration of substrate is gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, there will not be any increment in the rate of reaction. Allosteric inhibitors are substances that decrease the activity of enzyme through two ways; competitive inhibitors and noncompetitive inhibitors. Allosteric activators enhance the enzyme activity, and bid to allosteric sites in order to maintain the active configuration of enzyme. Cofactors are another important factor that affects the enzyme activity and it can be a coenzyme, a prosthetic group or a metal ion activator. Enzymes have broad-spectrum functions in the living organisms, contributing from signal transduction to generation of muscle contraction. They also catalyze the starch molecules into easily absorbed monomer unit by mammals called as maltose.

2. EFFECT OF ANAEROBIC PROBIOTICS ON FIBER STRUCTURE

Since the fibre fraction obtained from feces is fermentable, the degradation of fibre inside the rumen is not fully efficient [1]. In the last few years, researches focused on the supplementation of exogenous enzymes during *in vitro* rumen fermentation for fibre digestibility [2, 3]. However, the investigations still lack the positive influence of exogenous enzymes products on fibre digestibility. The ZAD® (a patented product manufactured by the Academy of Scientific Research and Technology, Egypt), a biotechnical product made from anaerobic bacteria converts the polysaccharide into monomers by the enzyme catalytic process. Gado, et al. [4] revealed that ZAD® improved nutrients digestibility, body weight gain and feed conversion of wheat straw in sheep. In fact, ZAD® is a mixture of enzymes obtained from anaerobic bacteria that had a beneficial impact on the digestibility of low quality roughages. According to Kingsly, et al. [5] DDGS are highly nutritious and valuable feed for animals due to the presence of high protein and energy content ingredient. However, variation in composition affects the nutritional quality and market value of the product [6].

Based on the findings, the rice straw (RS) depicted the highest (P<0.05) NDF and ADF contents with respect to the DDGS, while the CP content was the highest in DDGS. The inclusion of anaerobic enzyme (ENZ) at 3 L enhanced quadratically (P<0.01) the degradation of NDF and ADF of RS, and DDGS as well as their mixture (Table 1). Increasing the dose of ENZ from 0-3 L before ensiling RS, DDGS, and their mixture were known to improve quadratically (P<0.01) some of the degradation fractions of nutrients. In general, the inclusion of ENZ enhanced the degradable fractions of NDF and ADF of DDGS by 11%, the degradation fraction of NDF and ADF of RS plus DDGS by 6 and 8%, and the degradation rate of NDF and ADF of RS by 24% (Table 1) - Gado, et al. [7].

3. EFFECT OF ANAEROBIC PROBIOTICS ON DAIRY ANIMALS

Recently, researchers have demonstrated that the supplementation of fiber degrading enzymes into the feeding diets of dairy cows and feedlot cattle can improve feed utilization and animal performance by enhancing fiber degradation *in vitro* [8-11] *in situ* [12-15], and *in vivo* [9, 16-18]. There is often an increased feed intake due to the feeding of enzymes, which may partly be because of the increased palatability of the diet due to sugars released by pre-ingestive fiber hydrolysis. However, post-ingestive enzyme activities, such as increased digestion rate and/or extent of digestion [12, 15, 18, 19] may improve hydrolytic activity in the rumen in order to reduce gut fill and to enhance feed intake [20].

Several mechanism of action of direct-fed enzymes had been proposed such as dietary fiber solubilization before ingestion, provision of readily fermentable substrate for ruminants, and/or improvement of microbial enzyme activity in the rumen [21]. The enzyme efficacy is influenced by the specific activity of enzymes, their mode of application, type of animal, and feeding diet. Direct-fed enzymes can also improve the microbial colonization of feed by increasing total counts of ruminal microorganisms [22, 23] in order to increase fiber degradation rate [14, 16, 24] and protein synthesis of rumen microbes [16, 25].

Table-1. Effect of ENZ levels on *in vitro* degradation† of DM and fibre fractions of RS, DDGS, and their mixture in sheep (Adapted from Gado, et al. [7])

		ENZ, L ton^{-1}			SEM‡	Contrast§	
		0	1	3		L	Q
RS	DM						
	a (%)	11.0	17.3	22.6	4.66	0.006	0.024
	b (%)	33.4	39.2	44.7	3.12	0.009	0.035
	a+b (%)	44.4	56.5	67.3	2.88	0.006	0.041
	c (%/h)	2.3	3.4	4.6	0.68	0.008	0.021
	NDF						
	b (%)	41.5	44.6	48.6	1.45	0.009	0.016
	c (%/h)	2.1	2.4	2.7	0.42	0.240	0.340
	ADF						
	b (%)	38.2	41.1	44.7	1.44	0.008	0.019
	c (%/h)	2.4	2.6	2.8	0.61	0.190	0.048
DDGS	DM						
	a (%)	28.7	29.4	31.4	1.44	0.005	0.036
	b (%)	46.1	49.8	53.4	2.62	0.009	0.042
	a+b (%)	74.8	79.2	84.8	1.89	0.007	0.026
	c (%/h)	3.8	4.0	4.9	0.79	0.007	0.042
	NDF						
	b (%)	45.4	47.4	51.5	1.93	0.008	0.035
	c (%/h)	3.2	3.5	3.9	0.53	0.008	0.012
	ADF						
	b (%)	49.9	50.6	55.6	1.99	0.009	0.043
	c (%/h)	3.4	3.5	3.9	0.29	0.006	0.051
RS with 10% DDGS	DM						
	a (%)	15.6	26.4	33.8	3.73	0.009	0.043
	b (%)	34.2	46.3	55.1	2.47	0.008	0.031
	a+b (%)	49.8	72.7	88.9	4.83	0.008	0.026
	c (%/h)	3.2	4.3	5.4	0.29	0.006	0.04
	NDF						
	b (%)	42.5	48.6	53.6	3.81	0.007	0.042
	c (%/h)	2.8	3.1	3.6	0.33	0.006	0.043
	ADF						0.036
	b (%)	41.8	42.2	45.8	1.69	0.008	
	c (%/h)	2.6	3.1	3.4	0.29	0.006	0.046

†a, soluble fraction; b, potentially degradable fraction; a+b, total degradation; c, degradation rate. ‡SEM, standard error of the mean, §Probability of a linear (L) or quadratic (Q) effect of ENZ level.

The supplementation of exogenous enzymes to feeding diets has been known to exhibit positive influence on lactating dairy cows and growing cattle. Dairy cows fed with fibrolytic enzyme treated forage produced 5–25% more milk [14, 26, 27], increased the energy balance of transition dairy cows [28], and increased the production of milk in small ruminants [27, 29]. The treatment of fibrolytic enzymes improved live weight (LW) gain and feed conversion ratio by up to 35% and 10% respectively in feedlot cattle [30].

The ruminal fermentation, nitrogen balance, nutrient digestibility, and milk production of cows have been improved due to the addition of commercially available ZADO® [9, 31]. Further, the LW gain and feed conversion

of wheat straw in sheep and goats were also observed to be improved [17, 18]. Intake of DM and OM was positively affected by the supplementation of enzyme (Table 2). The digestibility of DM, OM, aNDFom and ADFom was higher (P<0.05) in the ZADO® enzyme supplemented cows.

Table-2. Intake, whole tract nutrients digestibility, and nitrogen balance of the TMRa fed to dairy cows supplemented with (ZADO®) or without (CTRL) the exogenous enzymes mixture

	CTRL	ZADO®	SEM	Significance (P)
Intake (kg/d)				
DM	16.1	18.2	0.21	0.049
OM	14.1	16.4	0.14	0.048
aNDFom	7.1	7.4	0.23	0.192
ADFom	4.04	4.57	0.11	0.087
Digestibility (g/kg)				
DM	663	743	2.1	0.045
OM	667	741	2.9	0.047
aNDFom	418	584	2.8	0.049
ADFom	401	532	2.3	0.046
Nitrogen balance				
N intake (g/d)	1654	1870	54	0.07
Urinary N (g/d)	509	542	12.6	0.11
Fecal N (g/d)	659	711	14.3	0.17
N balance (g/d)	486	617	11.9	0.06

DM, dry matter; OM, organic matter; ADFom, acid detergent fiber; aNDFom, neutral detergent fiber. TMR: total mixed ration without (CTRL) or with (ZADO®)

Enzyme supplemented cows showed higher (P<0.05) SCFA and ammonia N concentrations (Table 3) pre-feeding (122 versus 111 mmol/100 ml; 126 versus 110 mg/l respectively) and at 3 h post-feeding (128 versus 119 mmol/100 ml; 67 versus 55 mg/l respectively). Further, the addition of enzyme caused increased acetate and propionate 3 h post-feeding. An increase in N was also observed due to the ZADO supplementation, whereas the increment in excretion of N in urine and feces in the ZADO group was observed non-significant. The N balance in the ZADO group was higher than that of control group, while microbial N synthesis was increased in the enzyme supplemented cows (220 versus 190 g/d; P<0.05).

Table-3. Ruminal pH, short chain fatty acids (SCFAs, total and individual), ammonia N concentrations (after 0 and 3 h of feeding); microbial nitrogen synthesis of the TMRa fed to dairy cows supplemented with (ZADO®) or without (CTRL) the exogenous enzymes mixture

	CTRL	ZADO®	SEM	Significance (P)
pH	6.1	5.9	0.24	0.41
Before feeding (0 h)				
Total SCFA (mmol/l)	111	122	2.1	0.34
Individual SCFA (mol/100 mol)				
Acetate (A)	61.0	64.8	1.30	0.05
Propionate (P)	17.8	18.1	0.83	0.13
Butyrate	11.3	11.9	0.81	0.24
A:P ratio	3.43	3.85	1.162	0.14
Ammonia N (mg/l)	55	67	0.37	0.04
Post-feeding (3 h)				
Total SCFA (mmol/l)	119.2	128	3.6	0.04
Individual SCFA (mol/100 mol)				
Acetate (A)	60.0	64.0	1.2	0.04
Propionate (P)	18.3	20.8	0.87	0.01
Butyrate	10.9	11.0	0.96	0.31
A:P ratio	3.28	3.08	0.070	0.01
Ammonia N (mg/l)	110	126	2.3	0.05
Microbial N (g/d)	190	220	9.6	0.04
Uric acid (mmol/d)	22.4	24.6	0.67	0.16
Allantoin (mmol/d)	308	304	10.4	0.26

TMR: total mixed ration without (CTRL) or with (ZADO®) the commercial exogenous enzyme mixture

In the recent years, researchers have demonstrated that the supplementation of fiber degrading enzymes into the feeding diets of cattle enhanced the degradation of fibers effectively, thereby improving the feed utilization as well as animal performance [9, 17, 18].

The solubilization of dietary fiber before ingestion, and provision of readily fermentable substrate for ruminal microorganisms and for improvement of microbial enzyme activity in the rumen are considered the most common modes of action of direct-fed enzymes [21]. The enzyme yield is known to be influenced by diversified factors viz. the specific activity of the enzymes, their mode and level of application, and type of animal and its diet. Direct-fed enzymes can also improve the microbial colonization of feed by increasing total counts of ruminal fibrolytic microorganisms [22, 23] by enhancing fiber degradation rate in the rumen [14, 24], microbial protein synthesis in rumen [16, 25], and forestomach digestibility. The addition of exogenous enzymes to ruminant feeds has shown positive impact on feedlot cattle. Further, the fibrolytic enzymes have improved the live weight gain by 35%, and feed conversion ratio by 10% [30].

4. EFFECT OF ANAEROBIC PROBIOTIC ON RABBIT

Rabbits play a crucial role in the supply of animal proteins for humans and occupy a pivotal position among non-ruminants. It can effectively utilize cellulose-rich feed in rations constituting less than 20% of grain [32] because of the suitability of its digestive system for high cellulose rich diets [33, 34]. Rabbit are categorized just below poultry in term of feed efficiency due to their simple biological properties, short breeding cycle, and high feed conversion efficiency rate [35]. A variety of enzymes have been used as potent additives in the feeding diets of non-ruminants, and they are well-known to improve the rate of absorption of nutrients in the intestines [33, 34, 36]. The beneficial impacts of the supplementation of enzymes attribute to a significant reduction in the viscosity of digesta in the intestine and the non starch polysaccharides present in the endosperm cell walls [37]. Additionally, the supplementation of cellulolytic enzymes to the diet of rabbits has a pronounced positive influence on the weight gain [38]. Since, the rate of digestion of fibre and starch in young rabbits is limited [33, 34, 39], the supplementation of enzymes increased the dietary digestion as well as performance of young rabbits on starter diets [33, 34, 40]. Similar observation was also reported in 30 d old rabbits weaned at 25 d [41]. Previously, researchers had demonstrated the mechanism of action of exogenous enzymes on various segments of the rabbit gut. Sequeira, et al. [42] showed a significant reduction in the gastric pH due to the addition of enzymes, while enzymes showed non-significant affect on the gastric, intestinal, and caecal contents, even in the period following early weaning [43].

Artificial insemination or natural mating helps to achieve the improved productivity in rabbits [32, 44]. Previously, the EZ0 rabbit bucks showed a lower sexual drive than the EZ-supplemented rabbit bucks, coincident with a linear increase in blood testosterone levels with ascending levels of the diet EZ additive. The reaction time of rabbit bucks was found to be much longer than 4.2s with New Zealand white×Chinchilla rabbit bucks [45] and 14-21s in Black Baladi and White New Zealand rabbit bucks [46] due to the testosterone and estradiol, which synergistically stimulates male sexual behaviour [47]. However, chemo investigation, frequency of mountings, and reduced mount latency may stimulate estradiol. The slightly higher blood levels of testosterone in EZ-supplemented rabbit bucks apparently enhanced reaction time, thereby confirming the low sexual drive due to decreased testosterone concentration [48]. The blood testosterone concentration in EZ -supplemented groups was higher than in the EZ0 rabbit bucks. The positive influence of the supplementation of exogenous EZ might be because of a stimulatory role of nutrients made available to the animal on testicular steroidogenesis, as improved nutrition enhances testicular functions, thereby stimulating the synthesis of testosterone [49]. The incorporation of EZ to rabbit buck diets remarkably increased sperm concentrations and total sperm counts, indicating that diets constituting the enzymatic complex had impact on the spermatogenesis. However, the addition of exogenous fibrolytic enzymes in the diets has improved the fibre digestion due to the growth and bioactive proteins of

cellulolytic ruminal bacteria [50, 51]. Moreover, the enzyme preparation in the feeding diets could have improved intestinal mucosal development, which increases nutrient digestibility, which in turn would promote nourishment of the sertoli cells and seminal fluid. Other researchers had documented a remarkable improvement in semen volume, and total sperm cells were observed after the ejaculation of rabbit bucks [52, 53]. There is a strong correlation between animal nutrition and spermatogenesis, sperm maturation, and male reproductive system development [54]. The availability of macronutrients and micronutrients for spermatogenesis was found to be improved due to the supplementation of EZ. This might be because of the enhanced endocrine activity of rabbit buck gonads, which created a favourable environment for spermatogenesis process. The beneficial impact of the EZ complex dietary addition on semen characteristics was expressed in the long term because the spermatogenesis process in rabbit bucks takes approximately 47 d [55]. The ejaculate volume observed in the rabbit bucks that received the highest EZ level was comparatively higher than the previous reports [56].

The supplementation of EZ showed not only improvement in the sperm motility but also reduction in the abnormal sperm and dead spermatozoa, thereby suggested that dietary manipulation using an EZ additive improved the proliferation of sperm cells. This response could be due to the effect of nutritional components on neuroendocrine pathways that controls reproduction [57]. A valid assessment for fertility of rabbit bucks was obtained through *in vitro* oestrus cervical mucus penetration test. Previous studies revealed a significant correlation between several sperm quality parameters in farm animals [58, 59]. The study showed that the sperm migration through cervical mucus was increased due to the EZ complex additives.

Semen preservation is still a limiting parameter for the extensive commercial application programs in rabbits [60]. The survival rate of rabbit sperm drastically decreased after 36 h, and its fertilizing potentiality diminished after 16 h of storage [61]. Further, the study revealed that the semen from rabbit bucks receiving the maximal level of the EZ preparation showed increased motility than semen from other EZ groups, after 3 d of refrigeration, which is against the finding of Carluccio, et al. [61]. This could be due to a greater nutrient availability for rabbit bucks, thereby presenting increased sperm cells vitality. The positive effect of improved nutrition of sperm motility in other mammals was also documented [62]. This is undoubtedly a pivotal step for the breeding management of rabbit bucks. Decrease in the mean GOT and GPT activity in the seminal plasma of EZ supplemented rabbit groups was observed. This could be mainly due to a membrane stabilizing property of nutrients made available by the EZ complex, causing lesser release of these enzymes in seminal plasma. The leakage of these enzymes proved to be an indicator of semen quality [63]. Testes and epididymides are considered to be the possible sources of these enzymes [64].

5. EFFECT OF ANAEROBIC PROBIOTIC ON POULTRY

Exogenous enzymes are well known for improving the feeding values of feedstuffs [65, 66]. However, it has also been documented that enzyme cocktail (carbohydrase and protease) enhanced the productivity [67] and digestibility of corn and soybean meal, which induces less viscosity for broilers [68, 69]. The utilization of exogenous enzymes as additives in the diets of broiler chicken has gained immense attention because of both environmental and economical concern [70]. The efficiency of several commercial enzyme products has been well stated, but there is still some vagueness in their mode of action [71]. Moreover, a number of findings reported that the digestibility of nutrients in broilers improved due to the supplementation of dietary enzymes [69, 70, 72]. Additionally, the incorporation of enzymes accelerated the development of the immune organs [73]. Authors hypothesized that the improvement of the nutrient digestibility might be reflected in enhancing immunity [74]. ZADO®), a commercial exogenous enzyme mixture product has been shown to improve ruminal fermentation, N balance and nutrient digestibility, milk yield [9, 50] live body weight gain (BWG), and feed conversion ratio [4, 18, 75]. Additionally, the dietary serine protease showed improved BW feed efficiency and digestibility of fat, protein [76], and amino acids [77].

In another report, total number of 225 one d-old Hubbard broiler chicks were given the basal diet supplemented with 0.1, 0.3, 0.5 and 0.7 kg ZADO®. ZADO® constitutes a mix of anaerobic bacteria as well as xylanases (2.3 U/g), cellulases (7.1 U/g), alpha-amylase (61.5 U/g), and protease (29.2 U/g) in a powder form, obtained through an anaerobic fermentation process [4, 50]. The supplementation of 0.5 kg ZADO® in the diets of broiler displayed positive impact on the body weight and the liveability of the broilers at the growing period than that of fed control diets. Further, the results showed a significant ($P \leq 0.05$) increment in the body weight at 21 day of age for the group supplemented with Z3 than other groups and the same trend was recorded at 35 day of age. The Z3 group showed better LBW ($P \leq 0.01$) than control, Z2, and Z4 groups. The groups supplemented with Z3, Z4, and Z2 showed improved daily weight gain than control group. According to the report of Kocher, et al. [78], the improved performance was observed due to the inclusion of enzyme cocktail (pectinase, amylase and protease) in corn-SBM-based diets for chicks. Cowieson, et al. [79] indicated that exogenous xylanase, amylase, protease, and phytase (Avizyme) can be used to maintain the performance of birds fed on a nutritionally rich feeding diet. Additionally, Cowieson and Ravindran [69] demonstrated that the supplementation of enzyme product (xylanase, amylase, and protease) into corn-SBM based broiler diets improved BWG and feed efficiency, without affecting the feed intake values. Further, authors also reported that the supplementation of enzyme cocktail can improve the energy and amino acid values of corn-SBM-based diets, thereby offering economic values to producers. The incorporation of enzymes in corn-SBM-based diets improved starch digestibility, improved access to cell contents, modified the intestinal microbiota, and improved protein solubility. In another report, Saleh, et al. [67] demonstrated that the commercial enzymes constituting carbohydrases and protease (Energex) improved the productivity (BWG and FCR) of broilers fed corn-SBM-based diets, without influencing the feed intake values. Zanella, et al. [80] demonstrated that the supplementation of xylanase, amylase, and protease improved the energy and amino acid digestibility of a corn-SBM-based diet for broilers. Additionally, Kalmendal and Tauson [70] reported the improved FCR due to the supplementation of the combination of xylanase and serine protease. Moreover, Gracia, et al. [72] demonstrated improved BWG and FCR by 4 to 9% in amylase-supplemented diet compared to the control diet. Remus, et al. [81] summarized the influence of a combination of xylanase, amylase, and protease on ileal digestibility of amino acids for 5 broiler trials and recorded a mean response value of about 2%.

The plasma protein profile of broilers was affected due to the dose dependent supplementation of ZADO® in feeding diets. Significant increment in the total protein and globulin content was estimated, while no significant differences were observed in the albumin content. These findings had influenced the A/G ratio, thereby reflecting improvement in broilers immunity. Further, the study showed significant improvement in the relative weight of the spleen and bursa due to the supplementation of 0.5kg ZADO® into corn-based diets, suggesting that the addition of enzyme stimulated the development of the immune organ. No significant differences in calcium and phosphorus levels were noticed. The concentration of plasma thyroid hormones differed significantly among various treatments. Additionally, ZADO® supplementation reduced the cholesterol level in plasma, indicating that the enzyme additives may contribute a decisive role in the lipid metabolisms of broiler.

6. EFFECT OF ANAEROBIC PROBIOTIC ON FISH

Certain enzymes are known to deactivate the anti-nutritional components, and improve the nutritive value of plant-associated proteins in the feeding diets of fish. Previous report demonstrated the feeding trial strategy in order to evaluate the impact of ZADO® (containing amylases, proteases, cellulases, and xylanases) supplementation on the survival, growth, and carcass characteristics in rabbitfish. The additives caused enhancement in the total weight gain, body weight gain, and specific growth rate of rabbitfish, thereby stimulating the growth performance as well as nutrient utilization.

7. EFFECT OF ANAEROBIC PROBIOTIC ON BEEF

The ZADO® treatment has been known to improve the daily voluntary DM intakes, rumen microbial N synthesis, and digestibility of all nutrients in beef. Further, the additive increased the (P<0.05) the rumen ammonia N and total volatile fatty acids (TVFA's) concentrations before and 3 h post-feeding. A significant increase in the Daily gain due to ZADO® supplementation was due to the positive impact on the nutrient intake and digestibility, ruminal fermentation, and microbial protein synthesis. Increased ammonia N concentration in cows may probably be due to the presence of protease. Previous reports demonstrated that the supplementation of enzyme to diets increased gain probably because of the improved digestibility as well as energy available for the growth [15, 16, 30].

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REFERENCES

[1] D. O. Krause, S. E. Denman, R. I. Mackie, M. Morrison, A. L. Rae, G. T. Attwood, and C. S. McSweeney, "Opportunities to improve fiber degradation in the rumen: Microbiology, ecology, and genomics," *FEMS Microbiology Reviews*, vol. 27, pp. 663-693, 2003. *View at Google Scholar | View at Publisher*

[2] A. N. Hristov, A. N. Hristov, C. E. Basel, A. Melgar, A. E. Foley, J. K. Ropp, C. W. Hunt, and J. M. Tricarico, "Effect of exogenous polysaccharide-degrading enzyme preparations on ruminal fermentation and digestibility of nutrients in dairy cows," *Animal Feed Science and Technology*, vol. 145, pp. 182-193, 2008. *View at Google Scholar | View at Publisher*

[3] A. Z. M. Salem, A. A. Hassan, M. S. Khalil, H. M. Gado, H. Alsersy, and J. Simbaya, "Effects of sun-drying and exogenous enzymes on nutrients intake, digestibility and nitrogen utilization in sheep fed Atriplex halimus foliages," *Animal Feed Science and Technology*, vol. 171, pp. 128-135, 2012. *View at Google Scholar | View at Publisher*

[4] H. M. Gado, A. Z. M. Salem, N. E. Odongo, and B. E. Borhami, "Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs," *Animal Feed Science and Technology*, vol. 165, pp. 131-136, 2011. *View at Google Scholar | View at Publisher*

[5] A. R. P. Kingsly, K. E. Ileleji, C. L. Clementson, A. Garcia, D. E. Maier, R. L. Stroshine, and S. Radcliff, "The effect of process variables during drying on the physical and chemical characteristics of corn dried distillers grains with solubles (DDGS) - plant scale experiments," *Bioresource Technology*, vol. 101, pp. 193-199, 2010. *View at Google Scholar | View at Publisher*

[6] R. L. Belyea, K. D. Rausch, T. E. Clevenger, V. Singh, D. B. Johnston, and M. E. Tumbleson, "Sources of variation in composition of DDGS," *Animal Feed Science and Technology*, vol. 159, pp. 122-130, 2010. *View at Google Scholar | View at Publisher*

[7] H. M. Gado, A. Z. M. Salem, L. M. Camacho, and M. M. Y. Elghandour, "Influence of exogenous enzymes of ZAD on in vitro ruminal degradation of ensiled rice straw with DDGS," *Animal Feed Science and Technology*, vol. 13, pp. 303-308, 2013.

[8] A. N. Hristov, L. M. Rode, K. A. Beauchemin, and R. L. Wuerfel, "Effect of a commercial enzyme preparation on barley silage in vitro and in sacco dry matter degradability," *Proceedings Western Section American Society of Animal Science*, vol. 47, pp. 282–284, 1996.

[9] H. M. Gado, H. M. Metwally, H. Soliman, A. Z. L. Basiony, and E. R. El Galil, "Enzymatic treatments of bagasse by different sources of cellulase enzymes," presented at the 11th Conference Animal Nutrition, Al-Aqsor Aswan, Egypt on 2 November, 13–18, 2007.

[10] M. M. El-Adawy, A. Z. M. Salem, B. E. Borhami, H. M. Gado, M. S. Khalil, and A. Abo-Zeid, "In vitro cecal gas production and dry matter degradability of some browse leaves in presence of enzymes from anaerobic bacterium in NZW rabbit," presented at the The 9th WRSA World Rabbit Congress, Verona, Italy, June 10–13, 2008.

[11] M. A. M. Rodrigues, P. Pinto, R. M. F. Bezerra, A. A. Dias, C. V. M. Guedes, V. M. G. Cardoso, J. W. Cone, L. M. M. Ferreira, J. Colaco, and C. A. Sequeira, "Effect of enzyme extracts isolated from white-rot fungi on chemical composition and in vitro digestibility of wheat straw," *Animal Feed Science and Technology*, vol. 141, pp. 326–338, 2008. *View at Google Scholar* | *View at Publisher*

[12] P. Feng, C. W. Hunt, G. T. Pritchard, and W. E. Julien, "Effect of enzyme preparations on in situ and in vitro degradation and in vivo digestive characteristics of mature cool-season grass forage in beef steers," *Journal of Animal Science*, vol. 74, pp. 1349–1357, 1996. *View at Google Scholar* | *View at Publisher*

[13] G. E. Lewis, C. W. Hunt, W. K. Sanchez, R. Treacher, G. T. Pritchard, and P. Feng, "Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers," *Journal of Animal Science*, vol. 74, pp. 3020–3028, 1996. *View at Google Scholar* | *View at Publisher*

[14] J. M. Tricarico, J. D. Johnston, K. A. Dawson, K. C. Hanson, K. R. McLeod, and D. L. Harmon, "The effects of an aspergillus oryzae extract containing alpha-amylase activity on ruminal fermentation and milk production in lactating holstein cows," *Animal Science*, vol. 81, pp. 365–374, 2005. *View at Google Scholar* | *View at Publisher*

[15] N. A. Krueger, A. T. Adesogan, C. R. Staples, W. K. Krueger, S. C. Kim, R. C. Littell, and L. E. Sollenberger, "Effect of method of applying fibrolytic enzymes or ammonia to Bermuda grass hay on feed intake, digestion, and growth of beef steers," *Journal of Animal Science*, vol. 86, pp. 882–889, 2008. *View at Google Scholar* | *View at Publisher*

[16] W. Z. Yang, K. A. Beauchemin, and L. M. Rode, "Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows," *Journal of Dairy Science*, vol. 82, pp. 391–403, 1999. *View at Google Scholar* | *View at Publisher*

[17] A. Z. M. Salem, M. M. El-Adawy, H. Gado, and M. S. M. Khalil, "Feed intake, nutrient digestibility and animal growth performance in sheep and goats fed wheat straw," *ADSA PSA AMPA ASAS Joint Annual Meeting, San Antonio, TX, USA, July 8–12. Journal of Animal Science*, vol. 85, p. 107, 2007.

[18] H. M. Gado and A. Z. M. Salem, "Influence of exogenous enzymes from anaerobic source on growth performance, digestibility, ruminal fermentation and blood metabolites in lambs fed of orange pulp silage in total mixed ration," presented at the 59th Annual Meeting of the European Association for Animal Production, Vilnius, Lithuania, August 24–27, 2008.

[19] K. A. Beauchemin and L. M. Rode, "Use of feed enzymes in ruminant nutrition. In: Rode, L.M. (Ed.)," in *Proceedings Canadian Society of Animal Science Lethbridge, AB, Canada*, 1996, pp. 103–130.

[20] A. T. Adesogan, "Improving forage quality and animal performance with fibrolytic enzymes," presented at the In: Florida Ruminant Nutrition Symposium, 2005.

[21] T. A. McAllister, A. N. Hristov, K. A. Beauchemin, L. M. Rode, and K. J. Cheng, "Enzymes in ruminant diets. In: Bedford, M., Partridge, G. (Eds.), Enzymes in farm animal nutrition," presented at the CABI Publishing, Oxon, UK, 2001.

[22] D. P. Morgavi, K. A. Beauchemin, V. L. Nsereko, L. M. Rode, M. McAllister, and Y. Wang, "A trichoderma feed enzyme preparation enhances adhesion of fibrobacter succinogenes to complex substrates but not to pure cellulose," presented at the 25th Conf. Rumen Function, Chicago, IL, USA, 2000.

[23] V. L. Nsereko, D. P. Morgavi, L. M. Rode, K. A. Beauchemin, and T. A. McAllister, "Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms in vitro," *Animal Feed Science and Technology*, vol. 88, pp. 153–170, 2000. *View at Google Scholar* | *View at Publisher*

[24] L. A. Giraldo, M. L. Tejido, M. J. Ranilla, and M. D. Carro, "Effects of exogenous fibrolytic enzymes on in vitro ruminal fermentation of substrates with different forage:concentrate ratios," *Animal Feed Science and Technology*, vol. 141, pp. 306–325, 2008. *View at Google Scholar* | *View at Publisher*

[25] V. L. Nsereko, K. A. Beauchemin, D. P. Morgavi, L. M. Rode, A. F. Furtado, T. A. McAllister, A. D. Iwaasa, W. Z. Yang, and Y. Wang, "Effect of a fibrolytic enzyme preparation from trichoderma longibrachiatum on the rumen microbial population of dairy cows," *Canadian Journal of Microbiology*, vol. 48, pp. 14-20, 2002. *View at Google Scholar* | *View at Publisher*

[26] G. E. Lewis, W. K. Sanchez, R. C. Treacher, W. Hunt, and G. T. Pritchard, "Effect of direct-fed fibrolytic enzymes on lactational performance of midlactation Holstein cows," *Proceedings, Western Section, American Society of Animal Science and Canadian Society of Animal Science*, vol. 46, pp. 310–313, 1995.

[27] A. V. Stella, R. Paratte, L. Valnegri, G. Cigalino, G. Soncini, E. Chevaux, V. Dell'Orto, and G. Savoini, "Effect of administration of live saccharomyces cerevisiae on milk production, milk composition, blood metabolites, and faecal flora in early lactating dairy goats," *Small Ruminant Research*, vol. 67, pp. 7–13, 2007. *View at Google Scholar* | *View at Publisher*

[28] J. M. DeFrain, A. R. Hippen, K. F. Kalscheur, and J. M. Tricarico, " Effects of dietary α-amylase on metabolism and performance of transition dairy cows," *Journal of Dairy Science*, vol. 88, pp. 4405–4413, 2005. *View at Google Scholar* | *View at Publisher*

[29] H. Titi and W. F. Lubbadeh, "Effect of feeding cellulase enzyme on productive responses of pregnant and lactating ewes and goats," *Small Ruminant Research*, vol. 52, pp. 137–143, 2004. *View at Google Scholar*

[30] K. A. Beauchemin, L. M. Rode, and V. J. H. Sewaltm, "Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages," *Canadian Journal of Animal Science*, vol. 75, pp. 641–644, 1995. *View at Google Scholar* | *View at Publisher*

[31] M. S. Soliman, "Utilization of peanut hay in ruminant feeding," Ph.D. Thesis, Alexandria University, Alexandria, Egypt, 2006.

[32] S. Y. Saleh, K. A. Attia, M. Fouad, and M. M. Nassar, "Effects of multi-enzyme feed additive "kemzyme" or/and sodium bentonite "as a feed binder"on sexual activity and some fertility parameters of rabbit bucks," *Journal of Agricultural Science*, vol. 2, pp. 89-99, 2010. *View at Google Scholar* | *View at Publisher*

[33] N. Abdel-Aziz, M. El-Adawy, A. Z. M. Salem, M. A. Cerrillo-Soto, L. M. Camacho, and B. E. Borhami, "Effects of exogenous enzymes, lactobacillus acidophilus or their combination on feed intake, digestibility and performance of rabbits fed sugarcane bagasse," *Animal Feed Science and Technology*, vol. 14, pp. 137-145, 2014. *View at Google Scholar* | *View at Publisher*

[34] N. Abdel-Aziz, M. El-Adawy, M. A. Mariezcurrena-Berasain, A. Z. M. Salem, J. Olivares-Pérez, A. E. Kholif, and B. E. Borhami, "Effects of exogenous enzymes, lactobacillus acidophilus or their combination on feed performance response and carcass characteristics of rabbits fed sugarcane bagasse," *Journal of Integrative Agriculture*, vol. 14, pp. 544-549, 2015. *View at Google Scholar* | *View at Publisher*

[35] M. S. Hasanat, M. E. Hossain, M. P. Mostari, and M. A. Hossain, "Effect of concentrate supplementation on growth and reproductive performance of rabbit under rural condition," *Bangladesh Journal of Veterinary Medicine*, vol. 4, p. 129 132, 2006. *View at Google Scholar* | *View at Publisher*

[36] M. R. Bedford and A. J. Morgan, "The use of enzymes in poultry diets," *World's Poultry Science Journal*, vol. 52, pp. 61-68, 1996. *View at Google Scholar* | *View at Publisher*

[37] R. T. Zijstra, C. F. M. de Lange, and J. F. Patience, "Nutritional value of wheat for growing pigs: Chemical composition and digestible energy content," *Canadian Journal of Animal Science*, vol. 79, pp. 187-194, 1999. *View at Google Scholar* | *View at Publisher*

[38] S. Chandra, M. Mahender, M. G. Prakash, T. Raghunandan, and K. K. Reddy, "Productive performance of broiler rabbits fed diets supplemented with probiotic and enzymes under two systems of housing," *Indian Journal of Animal Research*, vol. 48, pp. 355-361, 2014. *View at Google Scholar* | *View at Publisher*

[39] M. Marounek, S. J. Vovk, and V. Skřivanová, "Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits," *British Journal of Nutrition*, vol. 73, pp. 463-469, 1995. *View at Google Scholar* | *View at Publisher*

[40] I. Gutiérrez, A. Espinosa, J. García, R. Carabaño, and C. De Blas, "Effect of levels of starch, fiber and lactose on digestion and growth performance of early-weaned rabbits," *Journal of Animal Science*, vol. 80, pp. 1029-1037, 2002. *View at Google Scholar* | *View at Publisher*

[41] C. Fernández, J. M. Merino, and R. Carabaño, "Effect of enzyme complex supplementation on diet digestibility and growth performance in growing rabbits," in *Proceedings of the Sixth World Rabbit Congress, 9-12 July, 1996, Toulouse, France*, 1996, pp. 163-166.

[42] J. Sequeira, N. Nicodemus, R. Carabaño, and M. J. Villamide, "Effect of type of wheat and addition of enzymes on some digestive parameters at different sampling time," in *Proc.: 7th World Rabbit Congress. 4-7 July, 2000, Valencia, Spain*, 2000, pp. 437-444.

[43] L. Falcão-e-Cunha, L. Castro-Solla, L. Maertens, M. Marounek, V. Pinheiro, J. Freire, and J. L. Mourao, "Alternatives to antibiotics growth promoters in rabbit feeding: A review," *World Rabbit Science*, vol. 15, pp. 127-140, 2007. *View at Google Scholar* | *View at Publisher*

[44] L. R. Rodríguez-De, M. Fallas-López, R. Rangel-Santos, V. Mariscal-Aguayo, P. A. Martínez-Hernández, and M. J. G. García, "Influence of doe exposure and season on reaction time and semen quality of male rabbits," in *Proc.: 9th World Rabbit Congress, June 10-13, 2008, Verona, Italy*, 2008.

[45] I. P. Ogbuewu, I. C. Okoli, and M. U. Iloeje, "Semen quality characteristics, reaction time, testis weight and seminiferous tubule diameter of buck rabbits fed neem (Azadirachta Indica A. Juss) leaf meal based diets," *Iranian Journal of Reproductive Medicine*, vol. 7, pp. 23-28, 2009. *View at Google Scholar*

[46] H. M. Safaa, M. E. Emarah, and N. F. A. Saleh, "Seasonal effects on semen quality in black Baladi and white New Zealand rabbit bucks," *World Rabbit Science*, vol. 16, pp. 13-20, 2008. *View at Google Scholar* | *View at Publisher*

[47] E. Cross and C. E. Roselli, "17 beta-estradiol rapidly facilitates chemo investigation and mounting in castrated male rats," *American Journal of Physiology*, vol. 226, pp. 497-509, 1999.

[48] A. E. B. Zeidan, I. F. M. Marai, and Z. A. Abd El-Kariem, "Effects of intratesticular injection of gonadotropin-releasing hormone on reproductive performance of low fertile male rabbits under Egyptian summer conditions," in *Proc.: 1st International Conference on Animal Production and Health, 2-4 September, 1997, Dokki, Egypt*, 1997, pp. 557-566.

[49] Y. A. Attia and K. I. Kamel, "Semen quality, testosterone, seminal plasma biochemical and antioxidant profiles of rabbit bucks fed diets supplemented with different concentrations of soybean lecithin," *Animal*, vol. 6, pp. 824-833, 2012. *View at Google Scholar*

[50] H. M. Gado, A. Z. M. Salem, P. H. Robinson, and M. Hassan, "Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows," *Animal Feed Science and Technology*, vol. 154, pp. 36-46, 2009. *View at Google Scholar* | *View at Publisher*

[51] Y. Wang, J. E. Ramirez-Bribiesca, L. J. Yanke, A. Tsang, and T. A. McAllister, "Effect of exogenous fibrolytic enzyme application on the microbial attachment and digestion of barley straw in vitro," *Asian-Australasian Journal of Animal Sciences*, vol. 25, pp. 66-74, 2012. *View at Google Scholar* | *View at Publisher*

[52] C. Castellini, P. Lattaioli, A. D. Bosco, A. Minelli, and C. Mugnai, "Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids," *Reproduction Nutrition Development*, vol. 43, pp. 91-103, 2003. *View at Google Scholar* | *View at Publisher*

[53] E. O. Ewuola, "Daily sperm production, gonadal and extra-gonadal sperm reserves of rabbits fed prebiotic and probiotic supplemented diets," *International Journal of Applied Agriculture and Apiculture Research*, vol. 9, pp. 48-53, 2013. *View at Google Scholar*

[54] Y. Cheah and W. Yang, "Functions of essential nutrition for high quality spermatogenesis," *Advances in Bioscience and Biotechnology*, vol. 2, pp. 182-197, 2011. *View at Google Scholar* | *View at Publisher*

[55] C. Boiti, C. Castellini, U. Besenfelder, M. Theau-Clément, L. Liguori, T. Renieri, and F. Pizzi, "Guidelines for the handling of rabbit bucks and semen," *World Rabbit Science*, vol. 13, pp. 71-91, 2005. *View at Google Scholar*

[56] D. Paál, J. Kročková, Ľ. Ondruška, T. Slanina, F. Strejček, and P. Massányi, "Effect of semen collection frequency on the progress in the motility of rabbit spermatozoa," *Slovak Journal of Animal Science*, vol. 47, pp. 61-67, 2014. *View at Google Scholar*

[57] G. B. Martin, J. Rodger, and D. Blache, "Nutritional and environmental effects on reproduction in small ruminants," *Reproduction Fertility and Development*, vol. 16, pp. 491-501, 2004. *View at Google Scholar*

[58] L. Richardson, J. P. Hanrahan, L. O'Hara, A. Donovan, S. Fair, M. O'Sullivan, S. D. Carrington, P. Lonergan, and A. C. Evans, "Ewe breeds differences in fertility after cervical AI with frozen-thawed semen and associated differences in sperm penetration and physicochemical properties of cervical mucus," *Animal Reproduction Science*, vol. 129, pp. 37-43, 2011. *View at Google Scholar* | *View at Publisher*

[59] C. Martínez-Rodríguez, M. Alvarez, L. Ordás, C. A. Chamorro, F. Martinez-Pastor, L. Anel, and P. de Paz, "Evaluation of ram semen quality using polyacrylamide gel instead of cervical mucus in the sperm penetration test," *Theriogenology*, vol. 77, pp. 1575-1586, 2012. *View at Google Scholar* | *View at Publisher*

[60] F. López-Gatius, G. Sances, M. Sancho, J. Yaniz, P. Santolaria, R. Gutiérrez, M. Núñez, J. Núñez, and C. Soler, "Effect of solid storage at 15°C on the subsequent motility and fertility of rabbit semen," *Theriogenology*, vol. 64, pp. 252-260, 2005. *View at Google Scholar* | *View at Publisher*

[61] A. Carluccio, D. Robbe, I. De Amicis, A. Contri, U. Tosi, F. Russo, and M. Paoletti, "Artificial insemination in rabbits: Laboratory and field trial with three different semen extenders," *World Rabbit Science*, vol. 12, pp. 65-79, 2004. *View at Google Scholar* | *View at Publisher*

[62] A. Contri, I. De Amicis, A. Molinari, M. Faustini, A. Gramenzi, D. Robbe, and A. Carluccio, " Effect of dietary antioxidant supplementation on fresh semen quality in stallion," *Theriogenology*, vol. 75, pp. 1319-1326, 2011. *View at Google Scholar* | *View at Publisher*

[63] N. S. Juyena and C. Stelletta, "Seminal plasma: An essential attribute to spermatozoa," *Journal of Andrology*, vol. 33, pp. 536-551, 2012. *View at Google Scholar*

[64] M. Kareskoski and T. Katila, "Components of stallion seminal plasma and the effects of seminal plasma on sperm longevity," in *Proc.: 5th International Symposium on Stallion Reproduction*, 2008, pp. 249-256.

[65] N. Mathlouthi, S. Mallet, L. Saulnier, B. Quemener, and M. Larbier, "Effect of xylanase and alpha-glucanase addition on performance, nutrient digestibility and physico-chemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet," *Animal Research*, vol. 51, pp. 395-406, 2002. *View at Publisher*

[66] R. Lázaro, M. García, M. J. Araníbar, and G. G. Mateos, "Effect of enzyme addition to wheat-, barley- and rye-based diets on nutrient digestibility and performance of laying hens," *British Poultry Science*, vol. 44, pp. 256-265, 2003. *View at Google Scholar* | *View at Publisher*

[67] F. Saleh, M. Tahir, A. Ohtsuka, and K. Hayashi, "A mixture of pure cellulase, hemicellulase and pectinase improves broiler performance," *British Poultry Science*, vol. 46, pp. 602-606, 2005. *View at Google Scholar* | *View at Publisher*

[68] O. A. Olukosi, A. J. Cowieson, and O. Adeola, "Age-related influence of a cocktail of xylanase, amylase and protease or phytase individually or in combination in broilers," *Poultry Science*, vol. 86, pp. 77-86, 2007. *View at Google Scholar*

[69] A. J. Cowieson and V. Ravindran, "Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: Growth performance and digestibility of energy, minerals and amino acids," *British Poultry Science*, vol. 49, pp. 37-44, 2008. *View at Google Scholar* | *View at Publisher*

[70] R. Kalmendal and R. Tauson, "Effects of a xylanase and protease, individually or in combination and an ionophore coccidiostat on performance, nutrient utilization and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet," *Poultry Science*, vol. 91, pp. 1387-1393, 2012. *View at Google Scholar* | *View at Publisher*

[71] M. R. Bedford, "The role of carbohydrases in feed stuff digestion," presented at the Poultry Feed stuffs: Supply, Composition and Nutritive Value. J. MacNab and N. Boorman, Ed. CAB International, Wallingford, UK, 2002.

[72] M. I. Gracia, M. J. Aranibar, R. Lázaro, P. Medel, and G. G. Mateos, "Alpha-amylase supplementation of broiler diets based on corn," *Poultry Science*, vol. 82, pp. 436-442, 2003. *View at Google Scholar | View at Publisher*

[73] F. Gao, Y. Jiang, G. H. Zhou, and Z. K. Han, "The effects of xylanase supplementation on growth, digestion, circulating hormone and metabolite levels, immunity and gut microflora in cockerels fed on wheat-based diets," *British Poultry Science*, vol. 48, pp. 480-488, 2007. *View at Google Scholar | View at Publisher*

[74] H. M. Safaa, S. A. Riad, F. R. Mohamed, S. S. Siam, and H. A. El-Minshawy, "Probiotic, prebiotic and yeast supplementation in broiler diets from 1 to 42 days of age: 2. Immune response and slaughter traits," *Poultry Science*, vol. 89, pp. 546-547, 2010.

[75] H. M. Gado, "Effect of enzymatic treatments for poor quality roughages on fiber digestibility and nitrogen metabolism in Baladi goats," *Egyptian Journal of Nutrition and Feeds*, vol. 1, pp. 50-56, 1997. *View at Google Scholar*

[76] D. M. Freitas, S. L. Vieira, C. R. Angel, A. Favero, and A. Maiorka, "Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease," *Journal of Applied Poultry Research*, vol. 20, pp. 322-334, 2011. *View at Google Scholar | View at Publisher*

[77] C. R. Angel, W. Saylor, S. L. Vieira, and N. Ward, "Effects of a mono-component protease on perfromance and protein utilization in 7- to 22-day-old broiler chickens," *Poultry Science*, vol. 90, pp. 2281-2286, 2011. *View at Google Scholar | View at Publisher*

[78] A. Kocher, M. Choct, G. Ross, J. Broz, and T. K. Chung, "Effects of enzyme combinations on AME of corn-SBM based diet in broilers," *Journal of Applied Poultry Research*, vol. 12, pp. 275-283, 2003. *View at Google Scholar | View at Publisher*

[79] A. J. Cowieson, D. N. Singh, and O. Adeola, "Prediction of ingredient quality and the effect of a combination of xylanase, amylase, protease and phytase in the diets of broiler chicks. 1. Growth performance and digestible nutrient intake," *British Poultry Science*, vol. 47, pp. 477-489, 2006. *View at Google Scholar | View at Publisher*

[80] I. Zanella, N. K. Sakomura, F. G. Silversides, A. Fiqueirdo, and M. Pack, "Effect of enzyme supplementation of broiler diets based on maize and soybeans," *Poultry Science*, vol. 78, pp. 561-568, 1999. *View at Google Scholar | View at Publisher*

[81] J. Remus, M. Hruby, and E. E. M. Pierson, "Impact of the Avizyme 1500 series on apparent ileal amino acid digestibility in poultry," *Poultry Science*, vol. 84, p. 11, 2005. *View at Google Scholar*