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Full Length Research Paper

Effect of an Enzymatic Complex of Protease Plus Xylanase and Nutrient Concentration in Diets on the Productive Performance of Broiler Chickens in the Dry Tropics

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The influence of an enzyme complex of protease+xylanase (ECPX) and nutrient concentration in diets on productive performance was assessed in 200, 1-day old mixed broiler chicks (53.3 ± 1.9 g) assigned at random in a 2^2 factorial design with 5 repetitions (10 chicks each). Factors were dietary level of nutrients (standard and low in nutrients) and ECPX (0 and 400 g/ton). The trial had two phases (starter and finisher) of 21 days each. Nutrient concentration was reduced in the low-nutrient diets considering the matrix of energy and protein released by ECPX (9.14% and 7.74% of CP; 47 and 53 Mcal/kg of ME, in starter and finisher, respectively). There was no interaction (P>0.10) between factors. The ECPX improved weight gain throughout the study (P <0.05) and feed intake in the finisher stage and total trial (P <0.01). Enzyme supplementation did not affect feed conversion (P> 0.10). Animals fed the standard diet had greater weight gain (P<0.05) and improved feed conversion throughout the study (P<0.05). It is concluded that in the dry tropics during the heat season the ECPX may enhance production performance of broilers fed both standard and low-nutrient diets. However, nutrient reduction alone may affect productive performance of broiler chickens.

Keywords: exogenous enzymes, nutrients, production, broilers, heat season.

INTRODUCTION

The biotechnological industry produces several enzymes with digestive properties at relatively low cost through fermentation with different microorganisms. When these enzymes (exogenous enzymes) are added in diets, nutrient absorption in the intestinal tract and productive parameters of broiler chickens are improved (Méndez-Domínguez *et al.*, 2009).

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The primary ingredients in rations for broiler chickens are cereal grains (corn, wheat, sorghum, etc.) and oilseed meals (soybean, canola, and others).Starch is the main digestible fraction of grains. The non-starch polysaccharides (NSP) contain glucans (cellulose) and heteropolysaccharides (hemicellulose formed by glucose, galactose and xylose), which have low hydrolyses in the digestive tract of poultry. The addition of exogenous enzymes in diets increases digestion and absorption of NSP, generating extra energy that is available for the production of broiler chickens (Cowieson and Ravindran, 2008). The protein fraction of cereal grains and oilseeds meals is not100% digestible by poultry. Exogenous enzymes with proteolytic activity are produced using microorganisms specifically selected for this purpose; these exogenous proteases have improved protein digestibility of feeds for broiler chicks. The use of different enzymes in poultry diets has increased digestibility (Carvalho *et al*., 2009; Bertechini *et al*., 2009) and growth performance of broiler chickens (Méndez-Domínguez *et al*., 2009).

Exogenous enzymes in diets of birds increase nutrient availability in the digestive tract, which must be considered in the formulation of diets for optimal use of ingredients and nutrients. If nutrients released by exogenous enzymes are not considered in poultry-diet formulation, then the surplus nutrients are not used by the animal, and are excreted. This has two main negative characteristics: 1) the unnecessary economic cost of enzymes, and 2) the increase in environmental pollution by the excreted nutrients. The genetic capability and outstanding growth performance of today's broiler chicken are well recognized; in some instances the nutrition and feeding may limit the production of broiler chicken. It has been shown that increasing nutrient concentration over standard diets improves growth performance of broiler chickens (Orduña-Hernández *et al*., 2016; Infante Rodríguez *et al*., 2016). Most research on exogenous enzymes in broiler chickens has involved diets reduced in nutrients without considering the additional nutrient requirements that today's broiler chicken might have by the genetic improvement. There are few reports considering standard diets plus exogenous enzymes for the dry tropics during the summer.

The objective of this study was to evaluate the growth performance of broiler chicks fed diets with different concentrations of nutrients, based on sorghum grain and soybean meal supplemented with an enzymatic complex of protease plus xylanase.

MATERIAL AND METHODS

The present study was carried out at the Facultad de Medicina Veterinaria y Zootecnia, of the Universidad Autónoma de Tamaulipas, located in Ciudad Victoria, Tamaulipas, in northeastern Mexico. The area is located at 23°44′ 06″N and 97°09′50″W, at an altitude of 340 m. The mean annual rainfall is 900 mm, and the average temperature is 25 °C (INEGI, 2006). These climatic characteristics are typical for dry tropics.

Two hundred,1-day-old mixed broiler chicks were used. The animals had an average body weight of 53.3 ± 1.9 g. The birds, of ROSS commercial genetic line, were randomly distributed into 4 treatments of 50 birds (5 replications or pens of 10 animals each). The birds stayed in floor pens throughout the study on beds of sorghum straw of 2 cm particle size. The animals had free access to water and feed during the entire experimental period. Feed intake, weight gain and feed conversion were recorded every 7 days.

Birds were vaccinated on day 1 of the study against fowl-pox by wing puncture and on day 7 against Newcastle and hepatitis using inclusion emulsified bodies subcutaneously. Chickens were raised in a manner similar to that conventionally used by commercial broiler producers.

Two stages of feeding were used: the first stage or starting, from 1 to 21 days of age, and second stage or finishing, from 22 to 42 days of age. Diets were formulated following NRC (1994) requirements for broilers, for the respective stages. Starting diets are shown in Table 1 and finishing diets are shown in Table 2.

The first factor considered was the enzyme complex protease + xylanase (ECPX) added to the feed at levels of 0 or 400 g/ton. The second factor was diet type, with a standard diet and a low-nutrient diet.

The 4 experimental treatments (T) were as follows: T1,standard diet without ECPX; T2,low-nutrient diet without ECPX; T3 standard diet with ECPX; T4 low-nutrient diet with ECPX. In the starter stage the metabolizable energy (ME) concentration was 3013and 2994kcal/kg, and crude protein of 21.47% and 19.51%,for standard and low-nutrient diets, respectively. In the finisher stage, the energy concentration was 3086 and 3033 ME kcal/kg, and CP of 18.90% and 17.47%,for the standard and low-nutrient diet, respectively.

The ECPX contained protease (activity of 75000 units / g) and xylanase (1000 units / g). The enzymes were obtained by fermentation with the microorganisms *Bacillus lincheniformis* and *Thermomyceslanuginosus*, respectively (RONOZYME® Blend 400 CT, DSM nutritional products).

Compared to the standard diets, the low-nutrient diets were reduced by 47 and 53 kcal/kg of ME for starter and finisher diets. Crude protein levels were reduced 1.96 and 1.43% in starter and finisher diets, which represents 9.14% and 7.74% less total protein for starter and finisher diets, respectively (Tables 1 and 2). The same adjustment was followed for lysine, considering the effect of nutrient released by the use of the enzyme complex (matrix of nutrients released by the enzyme complex).

Table 1. Experimental diets for the starter phase (% dry matter basis)

Ingredient	T1 Standard	T2 Low in nutrients	T3 Standard + ECPX	T4 Low in nutrients + ECPX
Sorghum, grain	59.4	67.4	59.36	67.4
Soybean meal	33.7	27.8	33.7	27.8
Vegetable oil	1.45	0.4	1.45	0.4
Tallow	1.45	0.4	1.45	0.4
Premix[a]	4.0	4.0	4.0	4.0
Enzyme complex	0	0	0.04	0.04
TOTAL	100	100	100	100
Calculated analysis				
ME, kcal/kg	3013	2966	3012	2964
CP, %	21.47	19.51	21.47	19.52
Lysine, %	1.17	1.01	1.17	1.01
Ca, %	0.95	0.94	0.95	0.94
P, %	0.71	0.69	0.71	0.69

[a] Concentrated premix for first phase broilers. Contains: calcium mono ortho phosphate, calcium carbonate, salt, growth promoter (BMD and 3-nitro), coccidiostat (sodium monensin), mineral oil, ethoxyquin, vitamin A acetate, vitamin D3, vitamin E acetate, vitamin K3, riboflavin (B2), vitamin B12, niacin, D-calcium pantotenate, choline chloride, BHT. Premix calculated to contain: calcium, 21.40 %; total phosphorus, 8.10 %; sodium, 3.40 %; L-lysine hydrochloride, 0.80 %; DL-methionine, 4.15 %.

Table 2. Experimental diets for the finisher phase (% dry matter basis)

Ingredient	T1 Standard	T2 Low in nutrients	T3 Standard + ECPX	T4 Low in nutrients + ECPX
Sorghum, grain	65.8	72.1	65.76	72.1
Soybean meal	26.8	22.4	26.8	22.4
Vegetable oil	1.55	0.6	1.55	0.6
Tallow	1.55	0.6	1.55	0.6
Premix[a]	4.0	4.0	4.0	4.0
Pigment	0.3	0.3	0.3	0.3
ECPX	0	0	0.04	0.04
TOTAL				
Calculated analysis				
ME, kcal/kg	3086	3033	3085	3032
CP, %	18.90	17.47	18.90	17.48
Lysine, %	0.95	0.85	0.95	0.85
Ca, %	0.94	0.93	0.94	0.93
P, %	0.68	0.68	0.68	0.68

[a] Concentrated premix for second phase broilers. Contains: calcium mono-ortho phosphate, calcium carbonate, salt, growth promoter (BMD and 3-nitro), coccidiostat (sodium monensin), mineral oil, ethoxyquin, vitamin A acetate, vitamin D3, vitamin E acetate, vitamin K3, riboflavin (B2), vitamin B12, niacin, calcium D-pantotenate, choline chloride, BHT. Premix calculated to contain: calcium, 19.80 %; total phosphorus, 7.30 %; sodium, 3.70 %; L-lysine hydrochloride, 4.33 %; DL-methionine, 5.15 %.

The variables examined were: average live weight gain, average daily feed intake and feed conversion (intake/gain); these variables were statistically analyzed at each feeding period, i.e. starter (1 to 21 d), finisher (22 to

Table 3. Main effects of treatments (enzymatic complex and diet type of diet) on the growth performance of broilers

	ECPX (g/ton)			Diet			Interaction	
	0	400	P =	Standard	Low in nutrients	P =	P =	SEM
Starter (1-21 d)								
Weight gain	727	768	0.049	781	713	<0.01	0.98	13.6
Feed intake	1162	1197	0.09	1187	1172	0.43	0.97	13.4
Feed conversion	1.61	1.56	0.21	1.52	1.64	<0.01	0.90	0.02
Finisher (22-42 d)								
Weight gain	1568	1669	0.03	1678	1560	0.01	0.53	28.8
Feed intake	3220	3382	0.01	3364	3238	0.04	0.11	39.0
Feed conversion	2.06	2.03	0.44	2.01	2.08	0.04	0.48	0.02
Total trial (1-42 d)								
Weight gain	2295	2437	0.01	2459	2273	<0.01	0.62	36.2
Feed intake	4382	4579	0.01	4551	4409	0.06	0.20	49.5
Feed conversion	1.91	1.88	0.26	1.85	1.94	<0.01	0.56	0.02

42 d) and total trial (1 to 42 d). An analysis of variance was performed in a completely randomized design with 2 x 2 factorial arrangement; both the main effects of factors as well as their interaction were analyzed. The considered factors were 2 levels of ECPX (0 and 400 g/ton) and 2 diets (standard diet and low-nutrient diet). In all cases significance was considered at a level of 0.05. Statistical analyses were performed using the statistical package SAS (2007).

RESULTS

The results of main effects of treatments (enzymatic complex and type of diet) on growth performance of broiler chickens are shown in Table 3. There was no interaction effect (P >0.10) between factors on the variables of growth performance of broiler chickens. Supplementation with ECPX improved weight gain of chickens in the starter (727 vs. 768 g; P = 0.05), and finisher stages (1568 vs. 1669 g; P = 0.03) and in the total trial (2295 vs. 2457 g; P = 0.01). It also improved feed consumption in the starting (1162 vs. 1197 g; P = 0.09), finishing (3220 vs. 3383 g; P = 0.01) and total trial (4382 vs. 4579 g; P = 0.01). The data are for animals in diets without and with ECPX, respectively. Enzyme supplementation did not influence (P > 0.10) feed conversion of broiler chicks.

Compared to the low-nutrient diet, the standard diet produced higher weight gain in broiler chicks at starter (781 vs. 713 g; P < 0.01), finisher (1678 vs. 1560 g; P = 0.01) and total trial (2459 vs. 2273 g; P < 0.01). Feed consumption was also improved in finisher (3364 vs. 3238 g; P = 0.04) and total test (4551 vs. 4409 g; P = 0.06), although at the starter stage, feed consumption was not influenced by type of diet (1187 vs. 1172; P = 0.43).

Broilers receiving the standard diet showed better feed conversion (feed consumption / weight gain) at starter (1.52 vs. 1.64; P < 0.01), finisher (2.01 vs. 2.08; P = 0.04) and total test (1.85 vs.1.94 for standard and low- nutrient diets, respectively; P < 0.01).

DISCUSSION

The absence of effect for the interaction (ECPX and type of diet) showed that for the starter and finisher stages, ECPX improved weight gain by 5.6 and 6.4%, respectively. Although broiler chickens gained more weight on diets with ECPX, they also had higher feed intake, producing no effect on feed conversion by ECPX. Consistently with this research, is reported (Cowieson *et al*., 2006) decreased productive performance of broiler chickens when nutrients were reduced in the diet; however exogenous enzymes (xylanase, amylase, protease and phytase) in the diet improved weight gain, although they observed improved feed conversion of chicks in both diets (standard and reduced in nutrients).Crude protein was reduced 2% with an energy reduction similar to that in the present study. These authors observed that broilers fed low in nutrient diet plus the exogenous enzymes may have similar growth performance than broilers fed the standard diet; they also observed that energy and P limit the growth of birds rather than N and Ca. While in the current study birds fed the low in nutrient diet, the exogenous enzymes improved growth performance, however did not reach the growth performance of the standard diet. A matching response is reported in production of broiler chicks fed diets based on

corn-soybean meal supplemented with an enzymatic complex of xylanase, amylase and protease (Cowieson and Ravindran, 2008). They reduced similar amounts of energy from the diet but reduced amino acids by 3%. Nutrient concentration did not influence feed intake or digestibility of nutrients, although the reduction in nutrients had negative effect on weight gain and feed efficiency. There was no interaction between diet and enzyme for either weight gain or FCR, suggesting similar beneficial responses regardless of the nutrient density of the diet.

For the current study supplementation of enzyme complex improved growth and feed intake of broiler chickens, although feed efficiency was not improved. Others have reported improved growth and feed efficiency of broiler chickens supplemented with exogenous enzymes (Dersjant-Li *et al.* 2015).

An increased feed intake and negative effect on feed conversion with energy (100 kcal/kg) reduction in the diet is reported in broiler chickens; but xylanase supplementation improved growth behavior of birds (O'Neill *et al*., 2012). This is different from the findings of the present study since feed intake was not influenced by nutrient reduction; although in agreement with present study, birds fed the standard diet had better weight gain and feed conversion than those fed the low-nutrient diet. Also, enzyme supplementation enhanced weight gain of broiler chickens in both the standard and low-nutrient diets. In contrast, the use of an enzymatic complex (more than one enzyme) not always produced positive effects on growth performance of broiler chickens. It was found (Barekatain *et al*., 2013) that xylanase supplementation improved feed efficiency, while protease addition enhanced BWG; however, the combination of xylanase and protease did not exhibit synergism on growth performance or nutrient utilization of broiler chickens. They also observed that xylanase (alone)increased degradation of NSP fraction increasing free sugars, and protease (alone) increased amino acid digestibility; however, supplementation with both enzymes did not improve digestibility of amino acids or non-starch polysaccharides, postulating the possibly of xylanase inactivation with the protease. Similarly, in wheat and soybean-based diets (Kalmendal and Tauson, 2012) was reported no effect on weight gain of chickens with supplementation of enzymes (proteases or xylanases); however, enzymes fed separately improved feed conversion and digestibility of starch, with no additive effect of enzymes on improvements in feed conversion. The efficacy of exogenous protease supplementation in diets for broiler chickens is reported by Erdaw *et al.* (2017) who described that up to 20% of soybean meal can be replaced by raw soybean meal in diets for broilers when microbial protease is supplemented.

The use of a non-starch polysaccharide degrading enzyme complex (xylanase, β-glucanase, and α-galactosidase) in low energy diets containing dried distillers grains with solubles has increased nutrient digestibility and improved growth performance of broiler chickens (Campasino *et al*., 2015). In agreement, Hahn-Didde and Purdum (2014) used xylanase, amylase and protease in moderate and low energy diets with DDGS in diets for laying hens and observed higher retention values for Ca, P, and CP across both phases and also had higher AME retention values for starting phase. In all their study, the reduction of ME, Ca, and P were not affected by enzyme complex supplementation.

It is reported (Min *et al*., 2011a) that broiler chicken weight was minimally affected by energy reduction (88 kcal/kg, ME); however amino acid reduction (5%) depressed body weight. In the same study no influence of enzyme supplementation (xylanases, beta-glucanases, pectinases, mannanases, phytase and alfa-galactosidase) was observed on growth performance. In another study (Min *et al*., 2011b) were formulated standard diets for broiler chickens based on corn soybean meal using DDGS (0 and 30% of diet) supplementing with or without enzyme complex (carbohydrase and phytase activity) at 1x, 2x and 4x times the recommended level. Enzyme supplementation did not influence body weight, feed intake, feed conversion or nutrient utilization. These results contrast with those obtained in the current study using diets based on sorghum and soybean meal with addition of xylanase and protease. This may have contributed to a greater benefit of the ECPX in the present study, since sorghum grain has a chemical bond of protein with starch, which makes it more resistant to enzymatic attack, but the use of the ECPX of xylanase and protease may have produced a better weight gain because of higher nutrient availability.

In the present study birds fed low-nutrient diets supplemented with ECPX approached the weight gain of those fed the standard diet without ECPX, while the lowest weight gain was for chicks fed the low in nutrients diet without ECPX. The highest weight gain was for broiler chicks fed the standard diet supplemented with the ECPX.

Consistently with the current study, Avila *et al.* (2012) also reported reduction in feed consumption with improved weight gain and feed conversion of broiler chickens fed low-nutrient diets based on sorghum and soybean when supplemented with a multiple-enzyme complex of carbohydrases and phytase. The authors did not consider a positive control (standard diet) with exogenous enzyme supplementation; in the present study the enzyme supplementation of standard diets also improved growth performance of broiler chickens. Present study was conducted in the heat season (15 July to 15 September) in a dry tropics, this condition could contribute to improve growth performance of birds with enzyme supplementation in both, standard an low and nutrient diets.

Most reports on exogenous enzymes in broiler chickens used corn and soybean meal-based diets and few of them

used sorghum instead of corn as in the present study. It has been reported that exogenous enzymes (amylases, xilanases and proteases) improve weight gain and feed conversion with corn grain in the diet; although weight gain was not increased using sorghum in the diet, feed efficiency of broiler chickens did improve (Cortés *et al.*, 2002).

In conclusion, during the heat season in the dry tropics the supplementation with enzymatic complex (protease + xylanase) may enhance the growth performance of broiler chicks fed diets based on sorghum and soybean meal, using both standard diets and low-nutrient diets; however the ECPX had greater positive effect on growth performance of broiler chickens with standard diets. In contrast, reduction in nutrients without ECPX is not recommended, particularly in the heat season.

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