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Enzymatic Programs for Broilers

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ABSTRACT

Two trials were conducted on floor pens to evaluate the performance of broilers fed with diets contained different carbohydrases enzyme programs at different ages. Trial 1- The body weight gain (BWG) was affected by the Negative Control (NC) treatment (P<0.05). However, when the birds were fed with the NC diet + Amilase (AMY)+Xilanase (XYL), the BWG improved and reached the same level as the Positive Control (PC). Trial 2- The birds received enzymes supplementation in the total phase and others only in the grower phase.The ME reduction by 120 kcal/kg increased the feed conversion ratio (FCR) (P<0.05) when compared to PC and none of the enzyme programs was able to recover the performance. It can be concluded that it is possible to supplement with AMY during the role period of broiler chicken life or use an enzymatic program with AMY or AMY+XYL only in the grower phase.

Key words: broilers, carbohydrase, enzymes, amylase, xylanase

INTRODUCTION

The use of enzymes in poultry nutrition has been studied for many years, although there was a significant increase in the number of recent publications. In several reports, the objective was to assess the action of a single enzyme or an enzyme mixture on the lifetime performance of broilers (Cowieson et al., 2003. Cowieson and Adeola, 2005, Juanpere et al., 2005, Mushtaq et al., 2007, Olukosi et al., 2007). Broilers, however, present physiological differences according to their age, size of gastrointestinal tract, production of endogenous enzymes, feed ingestion capacity, factors that will affect the digestibility of feed ingredients (Nitsan et al., 1991; Almirall et al., 1995; Uni et al., 1998; Geyra et al., 2001; Huang et al., 2005; Huang et al., 2006).

Data published by Leslie et al. (2007) suggested that there is a relationship between age and capacity of the digestive tract, which should be taken into account when enzymes are used in poultry diets. Cowieson et al. (2006) also found that enzymes have different actions in the development of broilers when the finisher phase is compared to the starter period. Another point to be taken into account are the non-starch polysaccharides (NSPs) and starch present in the feed when the starter and grower diets are compared, even when they are corn and soybean meal based. Besides having high starch levels, corn also has more than 5% of arabinoxylans

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(Choct, 2006). As corn is included at higher levels in the finisher diets for broilers, the ratio of these polysaccharides (starch and arabinoxylans) increase while other NSPs decrease when compared to the starter feed. No report on the use of different enzymes supplementing feed for the various broiler production stages was found. Thus, the objective of this study was to determine the possibility of using enzyme programs with different carbohydrases in corn and soybean meal based diets for broilers at different ages.

MATERIALS AND METHODS

All procedures were approved by the Ethics Committee on the use of animals in trials at the State University of Maringá (Report n° 031/2007, Protocol 012/2007). Two trials with broiler chickens in floor pens were carried out in a conventional poultry house covered with ceramic roof tiles, closed by wire netting, movable curtains, and concrete floor. The rice hull litter was changed after each trial, and feed and water were available *ad libitum*.

Table 1- Carbohydrates composition (%) of a starter and grower feed

Carbohydrates	Starter Feed[1]	Grower Feed [2]	Grower: Starter Ratio
Starch	33.47	38.78	1.16
Rhamnose	0.167	0.128	0.77
Fucose	0.097	0.078	0.81
Arabinose	1.763	1.682	0.95
Xylose	1.811	2.038	1.13
Mannose	0.622	0.552	0.89
Galactose	1.692	1.406	0.831
Glucose	3.257	3.025	0.929
Total NSPs	0.167	0.128	0.767
Uronic Acid	9.41	8.734	0.928

[1] – Considering a starter feed composition of 56% corn and 36% soybean meal.
[2] – Considering a grower feed composition of 64% corn and 29% soybean meal.

All feeds were based on corn and soybean meal and used as mashed feed. Samples of corn and soybean meal used in Trial 2 were submitted to the Novozymes Laboratory in Denmark to determine the non starch carbohydrates and starch composition of these ingredients. Based on these results, the carbohydrates composition was calculated for a starter feed (56% corn and 36% soybean meal) and a grower feed (64% corn and 29% soybean meal) (Table 1). It was found that starch and xylose had a 18% and 15% increase, respectively, and all the other NSPs and free sugar decrease when the grower diet was compared to the starter diet, in agreement with the assumption previously stated.

All diets used in Trial 2 were supplemented with phytase enzyme (the nutritional matrix values for phytase were 0.1% available phosphorus, 0.1 % Ca, and 12 kcal ME/kg) and was not considered as part of the treatment.

The feeding program included a starter feed and a grower feed, and performance was measured when the starter feed was replaced by the grower feed and also at the end of the experimental period. Body Weight Gain (BWG), Feed Conversion (FC), ratio between the total feed consumed and the sum of the birds live weight and the weight of the dead birds, Feed Intake (FI), and Body-weight Adjusted Feed Conversion (AFC) were measured in every stage and mortality was measured only at the end. The equations used to calculate AFC were based on Carvalho (2001) and the following formulas were used: in the starter phase, a 0.7 kg standard weight gain was used: AFC= (0.7-WG)+FC; in the grower phase, a 1.8 kg standard weight gain was used: AFC=[(1.8-WG)/3.2]+FC; and for the total experimental period a 2.5 kg standard weight gain was used: AFC= [(2..5-WG)/3.7]+FC. The 3.2 and 3.7 values present in the grower and the total experimental period formulas, respectively, are the correction factors for the standard weight gain. The WG and FC used in the formulas are the WG and FC for each phase. AFC is very useful in the FC comparison when there are different WG, allowing the comparison between different treatments and different trials.

The enzymes used in this trial were α-amylase (AMY), xylanase (XYL) and β-glucanase (GLU) and they were supplied by DSM Nutritional Products - Holland. The products and doses used were 400 ppm Ronozyme® A (CT) (containing α-amylase and endo 1,3:1,4-β-glucanases); 100 ppm Ronozyme® WX (CT) (containing endo-1,4-xylanase); and 360 ppm Ronozyme® VP (CT) (containing endo-1,3(4)-β-glucanase, pentosanase, hemicellulase and pectic-substance hydrolyzing activities), respectively. The doses that were used were based on the manufacturer's commercial recommendations and according to Vieira et al. (2007).

Trial 1 – Performance from 21 to 40 days of age
This trial was carried out to determine the possibility of using one or two enzymes only after the birds were 21 days of age.
A total of 680 male Cobb Vantress® - USA chicks was used in a fully randomized design, with four treatments and five replicates, 34 birds each. The birds were raised from 1 to 20 days of age under the same environmental conditions, and the starter feed was not supplemented with any exogenous enzyme. The following treatments were used: positive control (PC) with adequate nutritional levels, negative control (NC) with adequate nutritional levels except for metabolizable energy, which was reduced by 120 kcal ME/kg feed when compared to PC. The same feed used in the NC treatment was supplemented with AMY in third treatment and with AMY+XYL in fourth treatment. The composition and nutrients supplied by the feed in treatments PC and NC are presented in Table 2.

Table 2 - Grower feed composition used in Trial 1 (g/kg, as fed basis).

Ingredients	Positive Control	Negative Control
Corn	640.02	666.24
Soybean meal	283.03	278.28
Soybean oil	37.71	16.18
Salt	3.50	3.50
Sodium bicarbonate	1.03	1.02
Limestone	11.09	11.15
Dicalcium phosphate	16.54	16.46
L-Lysine HCl	2.04	2.13
DL-Methionine	2.50	2.48
L-Threonine	0.54	0.56
Mineral and Vitamin Premix[1]	1.50	1.50
Inert[2]	0.500	0.50
Calculate nutritive value		
CP (%)	18.50	18.50
AME (kcal/kg)	3,150	3,030
Dig. Lys (%)	1.04	1.04
Dig. Met+Cys (%)	0.77	0.77
Dig. Thr (%)	0.66	0.66
Ca (%)	0.90	0.90
Av. P (%)	0.42	0.42
Na (%)	0.18	0.18

[1]Mineral and Vitamin Premix provided per kg of feed: 9,000 IU Vitamin A (as all-*trans* retinol); 1,600 IU Vitamin D_3 (as cholecalciferol) ; 14 mg Vitamin E (as DL – α – tocopheryl acetate); 1.5 mg Vitamin K_3; 1 mg Vitamin B_1; 4 mg Vitamin B_2; 1.8 mg Vitamin B_6; 12 µg Vitamin B_{12}; 30 mg Niacin; 9 mg Pantothenic Acid; 50 µg biotin; 300 mg Folic Acid; 30 mg Fe; 9 mg de Cu; 60 mg Zn; 60 mg Mn; 0,25 mg Se; 1 mg I. [2] The enzymes were supplemented by the inert substitution.

Trial 2 - Performance from 1 to 40 days of age, with the use of amylase "on top" during the starter phase

This trial assessed the response to the enzyme used "on top" (that is, when the enzyme was used in a feed without reducing energy or any other nutrient) in the starter phase of the positive control (PC) or when used "on top" in the grower phase of PC or was supplemented to the negative control (NC), formulated to be nutritionally marginal in relation to energy (ME 120 kcal/kg less than PC).

The composition and nutrients supplied by the feed in treatments PC and NC are presented in Table 3.

Table 3 - Starter feed composition of the positive control treatment and grower feed composition of the positive and negative control treatments used in Trial 2 (g/kg, as-fed basis).

Ingredients	Phases		
	Starter	Grower	
	Positive Control	Positive Control	Negative Control
Corn	584.45	648.83	675.84
Soybean meal	353.00	281.00	276.00
Soybean oil	25.00	34.00	12.00
Limestone	13.00	12.00	12.00
Dicalcium phosphate	12.30	11.10	11.00
Salt	4.00	3.50	3.50
Sodium bicarbonate	1.10	1.00	1.00
L-Lysine HCl	1.02	2.08	2.17
DL-Methionine	2.64	2.49	2.47
L-Threonine	0.29	0.55	0.57
Choline Chloride 60%	0.90	0.80	0.80
Coban 400[1]	0.30		
Coxistac 12%[2]		0.55	0.55
Starter Min/Vitamin Premix [3]	1.50		
Grower Min/Vitamin Premix [4]		1.50	1.50
Ronozyme P 5000 (CT)[5]	0.10	0.10	0.10
Inert [6]	0.40	0.50	0.50
Calculate nutritive value			
CP (%)	21.50	18.50	18.50
AME (kcal/kg)	2,980	3,150	3,030
Dig. Lys (%)	1.15	1.04	1.04
Dig. Met+Cys (%)	0.86	0.77	0.77
Dig. Thr (%)	0.75	0.66	0.66
Ca (%)	1.00	0.90	0.90
Av. P (%)	0.45	0.42	0.42
Na (%)	0.20	0.18	0.18

[1] Monensin sodium 40%

[2] Salinomycin 12%

[3]Starter Mineral and Vitamin Premix providing per kg feed: 9,000 IU Vitamin A (all-*trans* retinol); 3,000 IU Vitamin D_3 (cholecalciferol); 69 µg 25-OH-D_3; 200 mg Vitamin E (DL – α – tocopheryl acetate); 3.50 mg Vitamin K_3; 3 mg Vitamin B_1; 8 mg Vitamin B_2; 6 mg Vitamin B_6; 40 µg Vitamin B_{12}; 50 mg Niacin; 15 mg Pantothenic Acid; 200 µg Biotin; 1.50 mg Folic Acid; 50 mg Fe; 10 mg Cu; 50 mg Zn; 80 mg Mn; 400 µg Se; 1 mg I; 1 mg Co.

[4]Grower Mineral and Vitamin Premix providing per kg feed: same mineral and vitamin levels of the starter phase except Vitamin E (DL – α – tocopheryl acetate) 50 mg.

[5]Phytase 5000 FYT/g

[6] The enzymes were supplemented by the inert substitution.

A total of 2,304 male Cobb® Vantress® - USA chicks were used, in 64 floor pens in a fully randomized design. Two treatments were used from 1 to 21 days of age, with 32 replicates each with 36 birds each: a PC (without enzyme) and a PC+AMY. Eight treatments were used from 21 to 40 days of age, with eight replicates. Each treatment of the starter phase was further divided into four during the grower phase. The four treatments of the grower phase originated from the starter phase that did not include enzymes were: PC, NC with 120 kcal less than PC, NC+AMY; and NC+AMY+XYL. The other four treatments in the grower phase originated from the starter phase with amylase supplementation were: PC+AMY; PC+AMY+XYL; NC+AMY; NC+AMY+XYL.

The performance results were submitted to variance analysis using the GLM procedure from SAS® (SAS Institute, 1990) and averages were compared by the Tukey's test, at 5% significance level.

RESULTS AND DISCUSSION

Table 4 described the broilers performance fed with different enzymes programs used in Trial 1. The use of enzymes only during the grower phase in a diet with 120 kcal/kg less than the control group improved the weight gain, especially in the group treated with AMY+XYL (P<0.05) when compared to the negative control (NC).

Table 4 - Feed conversion ratio (FCR), body weight gain (BWG), feed intake (FI), and adjusted feed conversion (AFC) of broiler chickens fed with corn soy diet from 21 to 40 days of age (Trial 1).

Treatments	FCR	BWG	FI	AFC
Positive Control [1]	1.835±0.035	1.556±0.050 [a]	2.855±0.099 [ab]	1.926±0.040
Negative Control (NC)[2]	1.894±0.055	1.444±0.055 [b]	2.733±0.057 [b]	2.021±0.070
NC + AMY [3]	1.863±0.085	1.522±0.028 [ab]	2.910±0.097 [a]	1.952±0.112
NC + AMY+XYL[4]	1.852±0.042	1.601±0.029 [a]	2.964±0.088 [a]	1.930±0.043
CV (%)	3.05	2.95	3.02	3.67
P	0.3585	0.0003	0.0031	0.1350

[1]Positive control (PC) with optimal apparent ***metabolizable*** energy (AME) level.
[2]Negative control (CN) with 120 kcal AME/kg less than the PC.
[3]AMY – α-amylase supplemented by 400 ppm inclusion of Ronozyme A.
[4]XYL – xylanase supplemented by 100 ppm inclusion of Ronozyme WX.
[a, b]Values followed by different letter in the same column are significantly different by Tukey test (P<0.05).

Feed intake was affected by the enzyme supplementation, as supplemented birds had a higher feed intake than the NC (P<0.05).
FI were not modified by the treatments (P>0.05). The use of enzymes during the grower phase can improve the birds digestive capacity. The result being a higher feed intake and, as a consequence, a higher weight gain. Jorgensen et al. (1990) concluded that genetic selection favored broilers with a better feed conversion (FC), excluding the birds that consumed more feed than their digestive capacity.
Therefore, when feeds supplemented with exogenous enzymes that improve the digestive capacity of poultry are used, broilers are able to increase their feed intake.
One of the reasons for the improved response when xylanase is used with amylase is that the starch is better used as a result of α-amylase activity, and soluble and insoluble NSPs as arabinoxylans are also degraded in free sugar as arabinose and xylose (Choct et al., 2004).
The improvement in performance obtained by adding xylanase to amylase may also be due to an improvement in the digestibility of amino acids from the feed ingredients, as reported by Rutherfurd et al. (2007). Studying the effect of α-amylase plus xylanase in broilers fed corn and soybean meal based diets, these authors found a significant improvement in the digestibility of all amino acids with the true ileal digestibility method.
The performance parameters in Trial 2 are shown in Tables 5 and 6. FC and BWG were not affected when the positive control with adequate AME was supplemented with AMY, but there was a 10g increase (P<0.05) in FI in the starter period, demonstrating that there is an increase in feed intake when the digestive capacity of broilers is improved. In this case, however, the higher feed

intake did not result in an improved weight gain. Olukosi et al. (2007) did not find any improvement in the birds performance when they supplemented the corn and soybean meal feed with a mixture of xylanase, amylase, and protease at 21 days of age. Iji et al. (2003) did not find an effect on broilers performance when enzymes were supplemented in the starter period. Gracia et al. (2003), found an improved performance when a corn and soybean meal based diet given to broilers from 1 to 42 days of age was supplemented with α-amylase. When broilers are fed a theoretically perfect diet it is unlikely that they will show improved results when an enzyme is used "on top", as the bird is already demonstrating its full potential and there is little room for improved performance.

Considering the whole period (1 to 40 days), only FC and AFC were influenced (P<0.05) by the treatments, and the FC of the PC was 3.5% better than NC (Table 6). The improvement with enzyme supplementation was not more than 1% for FC and AFC, and 1.5% for WG when AMY was used as a supplement for the NC in the grower phase and for the PC in the starter phase (P>0.05). The response was similar to that found when the NC was supplemented with AMY+XYL in the grower phase and with no AMY supplementation in the starter phase.

The average AFC in the grower phase of Trials 1 and 2 was 1.96 and 1.73, respectively, and the enzymes response on the broilers performance was lower in Trial 2, probably due to the excellent performance the broilers had independently of the treatment.

Table 5 - Feed conversion ratio (FCR), body weight gain (BWG), and feed intake (FI), of broiler chickens fed with corn soy diet from 1 to 20 days of age (Trial 2).

Treatments	**FCR**	**FI**	**BWG**
Positive Control (PC)[1]	1.277 ± 0.015	1.021 ± 0.019 [b]	0.799 ± 0.015
PC + AMY[2]	1.283 ± 0.015	1.031 ± 0.022 [a]	0.805 ± 0.015
CV (%)	1.19	2.03	1.85
P	0.1018	0.049	0.1117

[1]Positive control (PC) with optimal apparent ***metabolizable*** energy (AME) level.. [2]AMY – α-amylase supplemented by 400 ppm inclusion of Ronozyme A. [a, b]Values followed by different letter in the same column are significantly different by Tukey test (P<0.05).

Table 6 - Feed conversion ratio (FCR), body weight gain (BWG), feed intake (FI), and adjusted feed conversion (AFC) of broiler chickens fed corn soy diet from 1 to 40 days of age (Trial 2)

Treatments in each phase		**FCR**	**BWG**	**FI**	**AFC**
Starter	**Grower**				
Positive Control (PC)[1]	PC	1.574 ± 0.027 [c]	2.689 ± 0.083	4.229 ± 0.100	1.523 ± 0.045 [bc]
PC	Negative Control (NC)[4]	1.630 ± 0.012 [a]	2.656 ± 0.083	4.330 ± 0.147	1.588 ± 0.023 [a]
PC	NC + AMY	1.621 ± 0.017 [ab]	2.669 ± 0.052	4.328 ± 0.084	1.576 ± 0.025 [a]
PC	NC + AMY +XYL	1.621 ± 0.027 [ab]	2.696 ± 0.073	4.369 ± 0.125	1.568 ± 0.036 [ab]
PC + AMY[2]	PC + AMY	1.587 ± 0.028 [bc]	2.724 ± 0.089	4.319 ± 0.082	1.510 ± 0.025 [c]
PC + AMY	PC + AMY + XYL[3]	1.568 ± 0.011 [c]	2.758 ± 0.054	4.324 ± 0.068	1.498 ± 0.023 [c]
PC + AMY	NC + AMY	1.615 ± 0.027 [ab]	2.694 ± 0.050	4.351 ± 0.134	1.562 ± 0.023 [ab]
PC + AMY	NC + AMY + XYL	1.622 ± 0.022 [a]	2.682 ± 0.052	4.350 ± 0.110	1.573 ± 0.024 [ab]
CV		1.39	2.55	2.53	1.880
P		0.0001	0.1199	0.3203	0.0001

[1] Positive control (PC) with optimal AME level.
[2] AMY – α-amylase supplemented by 400 ppm inclusion of Ronozyme A.
[3] XYL – xylanase supplemented by 100 ppm inclusion of Ronozyme WX.
[4] Negative control (CN) with 120 kcal AME/kg less than the PC.
[a, b, c] Values followed by different letter in the same column are significantly different by Tukey test (P<0.05).

CONCLUSION

It can be concluded that it is possible to supplement corn and soybean meal based feeds with AMY during the role period of broiler chicken life or use an enzymatic program with AMY or AMY + XYL only in the grower phase, resulting in improved performance. The study of new enzyme programs, with different enzymes for the various phases, opens a new area in the study of enzymes in poultry nutrition where the enzyme specificity, substrate diversity, and animal physiology are all correlated.

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RESUMO

Para avaliar o desempenho de frangos de corte alimentados com rações com diferentes programas de enzimas carboidrases, foram realizados dois experimentos. No experimento 1 as aves receberam suplementação de enzimas apenas na fase de crescimento foi observado redução no ganho de peso (GP) daqueles alimentados com a ração Controle Negativo (CN) ou seja, ração com redução de 120 kcal/kg. No entanto, quando os frangos receberam a ração CN + Amilase (AMI) + Xilanase (XIL) o GP foi semelhante encontrado no Controle Positivo (CP). No Experimento 2, algumas aves tiveram enzimas em todo período de criação e outras tiveram apenas na fase de crescimento. O tratamento CN apresentou pior conversão alimentar que o CP. Pode-se concluir que é possível suplementar com AMI todo o período de criação das aves ou utilizar um programa enzimático com AMI ou AMI + XIL somente na fase de crescimento, sem prejuízo ao desempenho das aves.

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