The effect of lipase, amylase and protease addition on growth performance and nutrient digestion in young broiler chickens

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Research has shown that newly hatched birds may be deficient in key digestive enzymes and that the immaturity of the digestive system may result in poor utilization of dietary nutrients. It has also been demonstrated that nutrient digestion rather than the ability to absorb nutrients seems to be the primary limiting factor. Therefore, dietary supplementation of enzymes not produced in sufficient quantities by young chicks (i.e., lipase, amylase, protease) has been investigated in our laboratory. In one study, no effect of lipase addition (100 U/kg diet) on growth performance and fat utilization in broilers (4-18 d) fed a wheat-soybean meal diet was noted. Apparent digestibility of fat averaged 77.6% in the ileum and was the same (77.6%) following lipase addition. Total tract digestibility of fat averaged 88.0% and was similar to that of the lipase supplemented diet (87.8%). A similar study was conducted to determine if amylase and protease addition would improve nutrient digestion during the first two weeks of growth. The corn-soybean meal diet was fed in a mash form. The experimental treatments included the control diet and the diets supplemented with either amylase (10,000 U/kg) and amyloglucosidase (10 U/kg) or amylase, amyloglucosidase and protease (4,000 U/kg). There was no effect of enzyme supplementation on growth performance. Body weight gain and feed efficiency averaged 91.6, 95.5, 97.1g and 1.41, 1.38, 1.38 for the control and the amylase or amylase plus protease supplemented diets, respectively, in week one of the experiment (1-7 d), and 235.9, 242.8, 240.0g and 1.43, 1.40. 141 in week two of the experiment. This was corroborated by similar ileal starch and protein digestibility values which averaged 96.8, 96.8, 96.9% and 83.9, 80.1 and 79.6%, respectively, for the control and the amylase or amylase plus protease supplemented diets. Total tract digestibility of starch averaged 97.8, 97.7, and 97.7% for the three diets and was followed by similar AME_n values of 3129, 3129, and 3106 kcal/kg diet. It could be concluded from this study that the digestive enzyme deficiency in young chickens may not be as pronounced as originally thought. Consequently, supplementation of young poultry diets with starch-, protein- and fat-hydrolyzing enzymes should be reconsidered.

Keywords: young broiler chicken; enzymes; lipase; amylase; protease

Introduction

Research has shown that newly hatched birds may be deficient in key digestive enzymes and as reported by Nitsan et al. (1991), Noy and Sklan (1995) and Jin et al. (1998), specific activities of lipase, amylase and trypsin rapidly increase up to 2-3 weeks posthaching. Consequently, it has been suggested that the immaturity of the digestive system of young chicks may result in poor utilization of dietary nutrients (Jin et al., 1998). It has also been demonstrated that nutrient digestion rather than the ability to absorb nutrients seems to be the primary limiting factor (Parsons, 2004). Therefore, dietary supplementation of microbial lipase, amylase or protease enzymes not produced in sufficient quantities by young broiler chickens have been investigated in our laboratory.

Materials and methods

EXPERIMENTAL DESIGN AND DIETS

Two experiments were conducted to evaluate the effects of lipase (Experiment 1) or amylase and protease (Experiment 2) addition on growth performance and nutrient digestion in broiler chickens. The two basal diets used in the study (*Table 1*) were formulated to meet 95% NRC (1994) requirement for AME and 92% for crude protein, calcium, available phosphorus, methionine, and methionine + cystine. Other nutrients met or exceeded the NRC specifications. In Experiment 1, the lipase preparation supplied 100 units of activity per kg diet. In Experiment 2, the diets were supplemented with either amylase (10,000 U/kg) and amyloglucosidase (10 U/kg) or amylase, amyloglucosidase and protease (4,000 U/kg). Enzyme supplements were provided by Canadian Bio-Systems Inc., Calgary, Canada. Each diet contained chromic oxide (3.0 g/kg) as a marker for the calculation of nutrient digestibility coefficients and AME_n content.

Table 1. Composition and calculated analysis of the basal diets (g/kg).

Ingredient	Wheat-soybean meal diet	Corn-soybean meal diet
Wheat	600.0	
Corn		531.0
Soybean meal	215.0	370.0
Canola meal	60.0	
Peas	30.0	
Canola oil	50.0	50.0
Limestone[1]	14.6	13.1
Dicalcium phosphate[2]	10.8	16.0
DL-Methionine	0.8	
L-Lysine-HCL	0.8	
Mineral premix[3]	5.0	5.0
Vitamin premix[4]	10.0	10.0
Chromic oxide	3.0	3.0
Total	1,000.0	1,000.0
Calculated analysis		
CP (%)[5]	21.0	21.0
AME_n (kcal/kg)	3040	3052
Lysine (%)	1.15	1.12
Methionine (%)	0.46	0.56
Methionine + cystine (%)	0.86	0.91
Calcium (%)	0.92	0.95
Available phosphorus (%)	0.41	0.42

[1]Contained 38% calcium.
[2]Contained 21% calcium and 18% phosphorous.
[3]Mineral premix provided per kilogram of diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.36 mg; Na, 1.6 g.
[4]Vitamin premix provided per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 1,000 IU; vitamin E, 11 IU; vitamin B_{12}, 0.012 mg; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1,020 mg; folic acid, 0.75 mg; biotin, 0.25 mg; riboflavin, 5.5 mg.
[5]Calculated based on analyzed values of feed ingredients.

GROWTH PERFORMANCE

One-day-old male Arbor Acres broiler chicks were obtained from a local commercial hatchery. The birds were held in electrically heated Jamesway battery brooders for a 4-day pre-experimental period

(Experiment 1) or were assigned to the experimental treatments immediately upon arrival (Experiment 2). The birds were individually weighed and sorted into five weight classes. Groups of five birds were then randomly assigned to pens such that the average initial body weight was similar across pens. Ten replicate pens of five birds each were randomly assigned to the dietary treatments. All diets were fed in a mash form throughout the 2-wk experimental period. The birds had free access to water and feed and were provided with continuous light. Body weight (BW) and feed intake were monitored weekly with pen as the experimental unit. Prior to weighing, the birds were fasted for 4 h. Mean feed intake, BW, and feed efficiency were used to determine the growth performance.

NUTRIENT UTILIZATION

At the termination of the experiments, excreta samples from each pen were collected over a 3 h period and subsequently frozen, freeze-dried, and finely ground. The samples were analyzed for chromic oxide, gross energy, nitrogen, and fat contents. The total tract digestibility of fat and AME_n content of experimental diets were calculated. Following excreta collection, 20 birds from each treatment were randomly selected, killed by cervical dislocation and the contents of the ileum (from Meckel's diverticulum to 1 cm above the ileo-caecal junction) were collected. The digesta samples were frozen, freeze-dried, ground and pooled to yield 5 replicate samples per treatment. The samples were analyzed for chromic oxide, nitrogen, starch, and fat to determine ileal nutrient digestibilities.

CHEMICAL ANALYSES

Diet, digesta and excreta samples were analyzed in duplicate for fat content using the AOAC (1990) method 920.39. Chromic oxide was determined using the procedure described by Williams et al. (1962). Nitrogen content was analyzed by the combustion method using the LECO Model FP 2000 combustion analyzer. Gross energy was determined using a Parr 1261 adiabatic bomb calorimeter. Starch was determined colorimetrically using a Sigma Glucose (HK) 20 kit and the procedure described by Aman and Hasselman (1984).

STATISTICAL ANALYSIS

Both experiments were set up as completely randomized designs and data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 1986) program. Means were separated by using Duncan's multiple range tests (Snedecor and Cochran, 1980). All statements of significance are based on probability of less than 0.05.

Results and discussion

EXPERIMENT 1: LIPASE STUDY

The results of the growth performance of broilers during the 2-wk experimental period are presented in *Table 2*. There was no effect of lipase addition on feed intake, body weight gain and feed efficiency. Lack of response from lipase supplementation was corroborated by similar fat digestibility in the ileum (*Table 3*). In addition, total tract fat digestibility averaged 88.0% and was identical to that of the lipase supplemented diet (87.8%). No difference in the AME_n content was observed.

Table 2. Effect of lipase addition on growth performance of broiler chickens (5-18 days) fed wheat-based diets.

Diet	Feed intake (g/bird)	BW gain (g/bird)	Feed/gain
Control	678	475	1.44
Lipase[1]	695	485	1.43
Pooled SEM	7.6	3.9	0.006
P	0.113	0.184	0.122

[1] 100 U lipase per kg diet

Table 3. Apparent ileal and total tract fat digestibilities and AME_n content of lipase supplemented wheat-soybean meal diet fed to broiler chickens.

Diet	**Fat digestibility (%)**		**AME_n (kcal/kg)**
	Ileum	**Total tract**	
Control	77.6	88.0	3115
Lipase[1]	77.6	87.8	3108
Pooled SEM	0.61	0.23	11.0
P	0.963	0.465	0.650

[1]100 U lipase per kg diet

As discussed in our earlier publication (Meng et al., 2004), the lack of response in chicken performance and fat digestibilities to lipase addition suggests that the insufficiency of pancreatic lipase production may not be a factor contributing to incomplete fat digestion. Examination of the published research data indicates that although the daily net secretion of lipase into the duodenum increases significantly as the bird ages (Noy and Sklan, 1995), the secretion of lipase when calculated per gram of feed intake is less dramatic (Uni et al., 1996), indicating that the lipase secretion in young birds may not be as inadequate as expected when their feed intake is considered (Sklan, 2001). Although some increase in diet AME content and fat digestibility with lipase supplementation has been reported (Al-Marzooqi and Leeson, 1999), lipase addition caused a significant reduction in feed intake and consequently lowered body weight gain. This was not observed in the current study as performance parameters were similar to the control diet and no reduction in feed intake was noted. It would appear that such an “anorexic effect” observed earlier may have been a consequence of the high inclusion rates of lipase, which in the current study was not the case since enzyme addition, when calculated per g of feed intake, accounted for approximately 30% of the endogenous lipase secretion to the duodenum of the young chicken.

This and earlier research from our laboratory (Meng et al., 2004) suggest that factors other than the level of pancreatic lipase activity (i.e., water-soluble NSP) may be responsible for incomplete fat digestion by young chickens when fed a practical wheat-based diet.

EXPERIMENT 2: AMYLASE AND PROTEASE STUDY

Results of the growth performance of broiler chickens fed corn-soybean meal diets supplemented with either amylase and amyloglucosidase or amylase, amyloglucosidase and protease is summarized in *Table 4*. There was no effect of enzyme supplementation on growth performance. Body weight gain and feed efficiency values were similar for the control and the amylase or amylase plus protease supplemented diets in both week one and week two of the experiment.

Table 4. Growth performance of broiler chickens (1-14 d) fed corn-soybean meal diet supplemented with amylase or amylase and protease enzymes.

Diet	**Week 1**			**Week 2**			**Overall**		
	Feed intake (g/bird)	**BW gain (g/bird)**	**Feed/ gain**	**Feed intake (g/bird)**	**BW gain (g/bird)**	**Feed/ gain**	**Feed intake (g/bird)**	**BW gain (g/bird)**	**Feed/ gain**
Control	128.4	91.6	1.41	336.9	235.9	1.43	463.3	327.6	1.42
Amylase[1]	131.6	95.5	1.38	340.2	242.8	1.40	471.8	338.3	1.40
Amylase+protease[2]	133.3	97,1	1.38	338.3	240.0	1.41	471.6	337.1	1.40
Pooled SEM	1.6	2.0	0.017	5.6	5.3	0.016	6.8	6.9	0.014
P	0.1127	0.1611	0.4381	0.9204	0.6654	0.4208	0.7514	0.4914	0.3745

[1] 10,000 U amylase and 10 U amyloglucosidase per kg diet
[2] 4,000 U protease per kg diet

Although similar apparent ileal starch digestibility values were observed (*Table 5*), both dry matter and protein digestibility decreased slightly for the amylase or amylase plus protease supplemented diets.

Total tract digestibility of dry matter and starch for the control and the enzyme supplemented diets were similar and were followed by almost identical AME_n values (*Table 6*).

Table 5. Apparent ileal dry matter, starch, and protein digestibilities in broiler chickens fed corn-soybean meal diets supplemented with amylase or amylase and protease enzymes (%).

Diet	Dry matter	Starch	Protein
Control	74.5[a]	96.8	83.9[a]
Amylase[1]	72.2[b]	96.8	80.1[b]
Amylase + protease[2]	72.5[b]	96.8	79.6[b]
Pooled SEM	0.5	0.2	0.8
P	0.0064	0.9840	0.0039

1 10,000 U amylase and 10 U amyloglucosidase per kg diet
2 4,000 U protease per kg diet
a,b Means within a column with no common superscript differ significantly ($P < 0.05$).

Table 6. Apparent total tract dry matter, and starch digestibilities and AME_n content of enzyme supplemented corn-soybean meal diets fed to broiler chickens

Diet	Dry matter (%)	Starch (%)	AME_n (kcal/kg)
Control	75.8	97.8	3129
Amylase	75.5	97.7	3129
Amylase + protease	75.1	97.7	3106
Pooled SEM	0.4	0.1	15.7
P	0.4738	0.4264	0.5129

1 10,000 U amylase and 10 U amyloglucosidase per kg diet
2 4,000 U protease per kg diet

In disagreement with earlier research (Noy and Sklan, 1995; Zanella et al., 1999) and our own data (Meng and Slominski, 2005), the present study demonstrated that starch digestibility in birds fed a corn-soybean meal diet was as high at the ileal level as when measured by excreta collection and no effect of enzyme supplementation was noted. In addition, the total tract starch digestibility value determined in the current study agreed well with those reported earlier for a corn-based diet fed to broiler chickens (Weurding et al., 2001, Meng and Slominski, 2005, Parsons, 2004). As starch is the most important dietary energy source, its high digestibility resulted in high AME_n values (*Table 6*), which slightly excided the intended values when formulating the diets (see *Table 1*). Contrary to earlier research indicating that very young chicks have reduced ability to digest soybean protein (Parsons, 2004), there was no effect of protease addition on protein utilization with the control treatment showing the highest protein digestibility values at the ileal level.

Conclusion

It could be concluded from this study that the digestive enzyme deficiency in young chickens may not be as pronounced as originally thought. Consequently, supplementation of young poultry diets with starch-, protein- and fat-hydrolyzing enzymes should be reconsidered.

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