Effects of Different Levels of Supplementary Alpha-amylase on Digestive Enzyme Activities and Pancreatic Amylase mRNA Expression of Young Broilers*

Zhengyu Jiang, Yanmin Zhou**, Fuzeng Lu, Zhaoyu Han and Tian Wang
College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, 210095, China

ABSTRACT : Four hundred and forty 1-day-old Arbor Acre broilers were fed commercial starter diets with 0, 250, 750 and 2,250 mg/kg of an alpha-amylase preparation from 1 to 21 days of age to investigate the effects of an exogenous enzyme on growth performance, activities of digestive enzymes in the pancreas and anterior intestinal contents and pancreatic amylase mRNA expression. Body weight gain (BWG) and average daily gain (ADG) increased linearly ($p<0.01$) with increasing levels of supplementary amylase but feed conversion ratio (FCR) was not affected. There was a negative quadratic change of protease and amylase in the small intestinal contents with the increase of supplementary amylase level. The activity of intestinal trypsin was also increased ($p<0.05$). Lipase was unaffected by amylase supplementation ($p>0.05$). The pancreatic protease, trypsin, and lipase were not affected by exogenous amylase levels. Consistent with the tendency for a linear depression of amylase activity, pancreatic α-amylase mRNA was down-regulated by dietary amylase supplementation. The present study suggested that oral administration of exogenous amylase affected activities of intestinal enzymes and the production of pancreatic digestive enzymes in a dose-dependent manner. (**Key Words** : Amylase, Broilers, Growth Performance, Enzyme Activity, mRNA)

INTRODUCTION

The improvement of growth performance and nutrient availability of broilers fed diets with exogenous enzymes has been well reported. The mode of action of enzymes has been reviewed (Bedford, 2000; Cowieson, 2005) and many of these studies emphasized the ameliorative effect of enzymes on anti-nutritive factors such as β-glucan, arabinoxylan and phytic acid (Sarıçiçek et al., 2005; Selle et al., 2007) with little consideration of the response of digestive enzymes.

Studies on the development of the gastrointestinal tract (GIT) indicated considerable changes to both morphology of digestive organs and enzyme production in birds after hatching. A tremendous increase in the secretion of amylase in chicks from 4 to 21 days was observed by Noy and Sklan (1995), and similar change patterns in chicks and poults were demonstrated by other researchers (Kulka and Duksin,

1964; Ashild and Jerry, 1989; Nitsan et al., 1991). Additionally, low starch digestibility of less than 86% was determined between 4 and 21 days and an appreciable amount of intact starch granules in the ileum was observed by microscopic analysis (Noy and Sklan, 1995; Bedford and Autio, 1996). These results suggested that secretion of pancreatic amylase from the immature pancreas during the post-hatching period may retard intestinal starch digestion, and consequently limit early growth. Thus, exogenous amylase supplementation to a starter diet may have beneficial effects on broiler chicks.

Ritz et al. (1995) and Gracia et al. (2003) reported significant improvement of performance with amylase supplementation to corn-soybean meal diets in turkeys and broiler chicks. Mahagna et al. (1995), however, found no effective response of broilers aged 1 to 14 days when fed a sorghum-soybean meal diet with amylase and protease supplementation. Variation may occur depending on many factors such as age, type of diet and enzyme dose (Bedford, 2000; Acamovic, 2001; Cowieson, 2005). Mahagna et al. (1995) observed some carry-over effects on intestinal trypsin activity after the cessation of enzyme supplementation. Cowieson et al. (2006) reported that some

Table 1. Composition and nutrients of basal diets

Ingredient	Composition (%)
Maize	61.50
Soybean meal (45% CP)	33.00
Limestone	1.26
Dicalcium phosphate	1.70
DL-methionine	0.14
Lysine-HCl	0.08
Salt	0.32
Sand	0.50
Vitamin premix[a]	0.50
Mineral premix[b]	1.00
Total	100.00
Nutrients level (calculated)	
Metabolizable energy (MJ/kg)	11.7
Crude protein	20.4
Lys	1.09
Met+cys	0.77
Calcium	0.95
Total phosphorus	0.64
Available phosphorus	0.41

[a] Vitamin premix provided per kilogram of diet: Vitamin A, 12,000 IU; Vitamin D$_3$, 3,000 IU; Vitamin E, 20 mg; Vitamin K$_3$, 1.3 mg; Vitamin B$_1$, 2.2 mg; Vitamin B$_2$, 8 mg; Nicotinamide, 1 mg; Choline, 400 mg; Calcium pantothenate, 10 mg; Vitamin B$_6$, 4 mg; Biotin, 0.04 mg; Fursultiamine, 1 mg; Vitamin B$_{12}$, 0.013 mg.
[b] Mineral premix provided per kilogram of diet: Fe, 80 mg; Cu, 7.5 mg; Mn, 110 mg; Zn, 65 mg; I, 1.1 mg; Se, 0.3 mg; Bacitracin Zinc, 30 mg.

exogenous enzymes were able to increase the secretion of some endogenous materials from the GIT of broilers. Thus, it is possible that supplementation of enzymes, especially at excessive levels, impedes the beneficial responses of broilers by direct interaction with the GIT or digestive enzyme system. Furthermore, information is lacking about regulatory mechanisms involved in changes of α-amylase synthesis and secretion as affected by exogenous amylase.

Therefore, this study was designed to investigate the possible dose-dependent responses of growth, digestive enzyme activities within pancreatic tissue and small intestinal contents, and also pancreatic amylase expression with exogenous α-amylase supplementation of different levels in a broiler starter diet.

MATERIALS AND METHODS

Birds and diets

A total of 440 day-old Arbor Acres broiler chickens were selected on the basis of close-weight and equal-sex from a commercial hatchery and randomly allocated into four groups with 5 replicates of 22 broilers for each. A commercial corn-soybean meal diet (Table 1) and the same diet supplemented with 250, 750 and 2,250 mg/kg of an amylase (EC 3.2.1.1) preparation were used for the study. The commercial diet was formulated according to the recommendation from the Premix manufacturer. Amylase preparation was produced from *Bacillus subtilis* containing

4,000 unit/g enzymatic activity as determined and defined by the enzyme manufacturer (Wuxi Enzyme Preparation Company, Wuxi, China, 214035). All diets were fed in mash form. Chicks were reared in cages with wire floors. They were allowed *ad libitum* access to feed and water with a daily lighting regimen of 24 h throughout the experiment. The initial house temperature of 32°C was reduced gradually according to the age of the birds until reaching 26°C at 21 days.

Sampling procedure

After the 21 day experimental period birds were weighed by replicate after starvation for 12 h, and feed consumption in each cage was recorded. Then one male chick per replicate was randomly selected and given each feed for 6 h before killing by cervical dislocation. The GIT was quickly excised. Pancreas and anterior intestinal (duodenal and jejunal) contents were removed, and frozen immediately in liquid nitrogen before being stored at -70°C and -20°C, respectively.

Digestive enzyme assay

Pancreas, after homogenization with ice-cold physiological saline, and intestinal contents were centrifuged for 15 min at 4°C at 9,000 rpm and 4,000 rpm, respectively. After centrifugation, aliquots of the supernatant were collected for enzyme assay, making appropriate dilutions where necessary. The pancreatic zymogens were activated with purified enterokinase (Sigma, 71K7665) using the procedure described by Glazer and Steer (1977) before determination of trypsin and protease.

All determinations were carried out in duplicate. Amylase activity was determined according to the methods of Somogyi (1960) using a kit (Cat. C016, Jiancheng Bioengineering Institute). The unit of activity was defined as the amount of enzyme that hydrolyzes 1.0 mg substrate per 3 min at pH 6.9 at 40°C. Protease activity was assayed according to a modified method of Brock et al. (1982), using casein as the substrate and reacting it with Folin reagent (Sigma, 0425S03). Trypsin activity was assessed according to the original method of Erlanger et al. (1961), as modified by Iwamori et al. (1997), using 1 mmol/L BAPNA (Sigma, 104K1372) as the substrate with a calibration curve obtained from ρ-nitroaniline (Sigma, 20K3454). The activities of protease and trypsin were expressed as units defined as the amount of enzyme that hydrolyzes specific substrate to form 1 μmol product per min under the assay conditions. Lipase was measured using a turbidimetric method as described by Verduin et al. (1973) and the activity was expressed as units defined as the amount of enzyme that hydrolyzes olive oil to form 1 μmol product per min. The method of Lowry et al. (1951) was used for protein determination. Specific activities of

Table 2. Effect of supplementary amylase on body weight gain, feed intake, and feed conversion in broilers

Item	Supplementary amylase levels (mg/kg)				SEM	Linear	Quadratic
	0	250	750	2,250			
Initial BW (g)	43.2	42.7	43.6	43.2			
1-21 days							
21d BW (g)	572	572	575	598*	3.4	<0.01	NS
ADFI (g)	43.9	44.0	44.5	45.6	0.3	<0.05	NS
ADG (g)	28.6	28.6	28.7	29.9*	0.2	<0.01	NS
FCR	1.54	1.54	1.55	1.53	0.011	NS	NS

* Means were significantly different in comparison with the control (p<0.05).
NS: no significance.
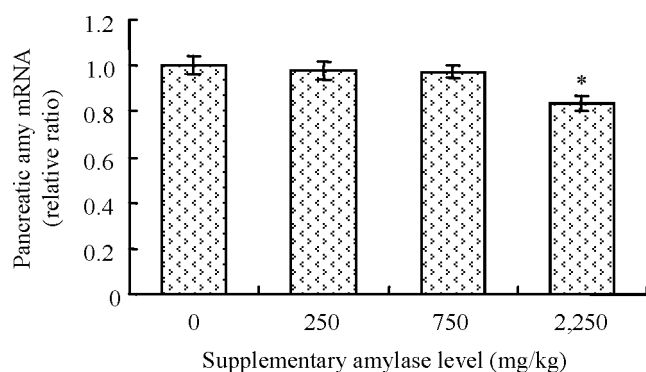

Figure 1. Quantification of pancreatic α-amylase mRNA in broilers fed diets supplemented with different amylase levels (n = 5). Data were presented as means±SE; linear, <0.01, quadratic NS; * Means were significantly different in comparison with the control (p<0.05).

pancreatic enzymes were expressed as the total activity per mg of digested protein.

Reverse transcription (RT) and Real-time quantitative PCR (Q-PCR) analysis

Total RNA was isolated from pancreas using TRIzoL reagent® (Invitrogen, 15596-026) according to the manufacturer's protocol. RNA integrity was electrophoretcally verified by ethidium bromide staining. Its purity was examined by UV spectrophotometry using the value of OD_{260}/OD_{280} and all samples were >1.80.

The reverse transcription of total RNA was carried out following the Promega protocol. Briefly, 3.0 μl of each aliquot of RNA, diluted with the addition of 2.0 μl of Oligo-dT_{15} and 5.0 μl of RNase in water, was heat-denatured at 70°C for 5 min, followed immediately by cooling on ice; after immediate centrifugation, 5.0 μl of M-MLV RT reaction buffer, 5.0 μl of dNTPs, 25 U of RNase inhibitor (Promega, N2511), 200 U of M-MLV transcriptase (Promega, M1701) and 3.5 μl of RNase in water was added to each sample to form a 25 μl reaction mix. The reaction mix was incubated at 42°C for 60 min and then heated at 90°C for 5 min to inactivate the reverse transcriptase. RT products (cDNA) were obtained and stored at -20°C for

quantification by real-time PCR.

Quantitative analysis of PCR was performed in the DNA Engine Opticon (MJ Research) using SYBR Green I (Generay, RS0976) as a double-stranded DNA-specific fluorescent dye. The amylase cDNA was amplified employing a pair of primers: (5'-GAC GGT CAG CCT TTC TCA-3') (forward, nt 1270-1287) and (5'-TAC ACG CAC TGC CTT CCT-3') (reverse, nt 1435-1452). The β-actin cDNA was amplified using primer pairs: (5'-TGC GTG ACA TCA AGG AGA AG-3') (forward, nt 695-714), (5'-TGC CAG GGT ACA TTG TGG TA-3') (reverse, nt 974-993). The amylase and β-actin cDNA primers were, respectively, designed based on sequences NM_001001473 and NM_205518 located in GenBank (http://www.ncbi.nlm.nih.gov/Entrez/index.html). For each amplification, 2 μl cDNA template was added to 23 μl master-mix containing 0.5 μl $MgCl_2$ (250 mM), 0.5 μl forward primer, 0.5 μl reverse primer, 0.75 μl dNTP (10 Mm), 0.25 μl Taq polymerase and 1.0 μl SYBR Green I. The optimized thermal cycling programs were 95°C for 1 min followed by 49 cycles of 95°C for 10 sec, 55.9°C or 55.3°C (for β-actin and amylase, respectively) for 20 sec and 72°C for 20 sec. One negative control was included in all reactions. Specificities of amplification products were confirmed by melting curve analysis (60.0°C to 95.0°C in 0.2°C/s increments).

The quantity of amylase mRNA in each sample was normalized by β-actin, and amylase cDNA was quantified using relative standard curve methods as described by Longergan et al. (2003). Since the amplification efficiency of target and references genes slightly differed, quantification of copy number of these genes was, respectively, derived from a different standard curve for target and references genes. The mean value of control group samples was assigned a value of 1 and normalized target values were expressed as percentage of the control group (Figure 1).

Statistical analysis

Data were analyzed using the General Linear Model (GLM) Procedure of SAS (SAS Institute, 1991). The nature of response to increasing levels of supplementary amylase

Table 3. Effect of supplementary amylase on activities of digestive enzymes in pancreas and intestinal contents

Item (n = 5)	Supplementary amylase levels (mg/kg)				SEM	Linear	Quadratic
	0	250	750	2,250			
Intestinal contents (U/ml)							
Amylase	268.3	318.3	439.3*	357.3*	19.7	NS	<0.01
Protease	929.8	1,329.2*	1,220.0*	1,144.5*	44.1	NS	<0.01
Lipase	3.67	3.52	3.69	3.41	0.07	NS	NS
Trpsin ($\times 10^{-1}$)	2.09	2.78*	2.51*	2.75*	0.08	NS	NS
Pancreatic tissue (U/mg protein)							
Amylase	295.7	274.4	272.1	182.7	21.9	0.06	NS
Protease	41.2	44.1	40.3	35.4	2.7	NS	NS
Lipase ($\times 10^{-2}$)	15.1	14.8	15.6	10.5	1.72	NS	NS
Trypsin ($\times 10^{-2}$)	1.48	1.51	1.73	1.30	0.09	NS	NS

* Means were significantly different in comparison with the control ($p<0.05$).
NS: no significance.

was determined by polynomial contrasts including linear and quadratic models. One-way analysis of variance was performed and differences between the supplementary amylase and control groups were detected using LSD test.

RESULTS

Performance

Increasing the level of supplementary amylase from 0 to 2,250 mg/kg increased BW, average daily feed intake (ADFI) and ADG linearly (Table 2). Supplementary amylase had no effect on FCR. Compared to the control, body weight was significantly enhanced with amylase supplementation at the level of 2,250 mg/kg ($p<0.05$).

Activities of digestive enzymes in pancreas and intestinal contents

The activities of amylase and protease in small intestinal contents showed negative quadratic responses to the increase in supplementary amylase level ($p<0.05$, Table 3). In comparison with the control, dietary amylase supplementation of increasing level significantly elevated the activities of intestinal protease and trypsin ($p<0.05$); dietary amylase at the level of 750, 2,250 mg/kg significantly increased the activity of intestinal amylase ($p<0.05$). Lipase was not affected by amylase supplementation at all levels ($p>0.05$). There was no significant effect of supplementary amylase on the activities of amylase, protease, trypsin, lipase in pancreas on a per milligram protein basis, but there was a linear trend to decrease the activity of pancreatic amylase.

Expression of pancreatic amylase mRNA

There was a negative linear relationship between pancreatic α-amylase mRNA level and supplementary amylase levels ($p<0.01$, Figure 1). Compared with the control, the diet supplemented with 2,250 mg/kg amylase significantly depressed pancreatic α-amylase mRNA

($p<0.05$).

DISCUSSION

Many researchers have reported increased body weight gain and improved feed conversion ratio as a consequence of amylase or enzyme combinations containing amylase being supplemented to poultry diets (Zanella et al., 1999; Piao et al., 1999; Iji et al., 2003; Gracia et al., 2003). It was generally believed that amylase improves productive performance by complementing the digestive system of the birds (Inborr, 1990). In the present study, oral administration of 2,250 mg/kg α-amylase significantly improved ADG by 4.5% and ADFI by 3.6%, which agrees with Ritz et al. (1995). Gracia et al. (2003) reported 9.4% improvement in BWG and 4.2% in FCR in 7-day-old broilers fed diets based on corn-soybean meal diet supplemented with a single exogenous α-amylase. However, Mahagna et al. (1995) found no beneficial effect of supplementary 250 or 1,000 μg/kg amylase and protease preparations on feed intake or growth rate from 1 to 14 days with a sorghum-soybean meal diet. Effects of exogenous enzymes on growth performance of broilers are variable. Gracia et al. (2003) attributed the discrepancies to the amylase origin (*Bacillus amyloliquefaciens* vs. *Bacillus subtilis*), amylase activity, cereal type (corn vs. sorghum), and feed form (mash vs. crumble). Alpha-amylase used in our study was produced from *Bacillus subtilis*, as reported by Mahagna et al. (1995), and supplemented to a corn-soybean meal based diet in mash form, as in the study of Gracia et al. (2003), whereas no significant improvement was observed on growth performance with different levels of supplementary amylase except for 2,250 mg/kg. Therefore, it is more reasonable to ascribe the discrepant effects to the supplementary dose of exogenous amylase.

Enzyme activity in the supernatant of anterior intestinal contents was of interest as an estimating criteria for endogenous enzymes, because most of the amylase (Osman et al., 1982), trypsin (Bird, 1971) and lipase (Rideau et al.,

1980) activities were distributed in the contents of duodenal or jejunal sections of the small intestine. The supplementation of amylase resulted in higher levels of intestinal amylase activity, which agrees with the results of Ritz et al. (1995) possibly due to an additive effect of exogenous and endogenous amylase. Mahagna et al. (1995) found that the activity of intestinal proteases was reduced when chicks were fed diets supplemented with exogenous amylase and protease. The present study, however, produced different results as we found significant increases of the activities of protease, trypsin in the anterior intestine with the supplementation of exogenous amylase. The results indicate that there may be beneficial effects of amylase supplementation on the secretion of proteolytic enzymes, but the positive responses of amylase and proteolytic enzymes were depressed with the amylase level of 2,250 mg/kg.

The activity of the pancreatic amylase was not significantly changed as a result of oral amylase administration, which is consistent with studies in poults (Ritz et al., 1995) and chicks (Nitsan and Madar, 1978). It is widely believed that the differences of physical structure between endogenous animal amylase and that of bacterial or plant origin may not result in feedback inhibition of pancreatic amylase production (Inborr, 1990; Ritz et al., 1995). In our study, however, the mean pancreatic level of amylase activity was depressed from 9% to 33% with increasing levels of exogenous amylase from 250 mg/kg to 2,250 mg/kg, which might indicate a possible negative response of pancreatic enzyme production.

Interestingly, decreases in pancreatic amylase activity were associated with a response in amylase mRNA expression. This suggests that presence of exogenous amylase in the intestine influences the pancreatic amylase at the transcriptional level. In rats, dietary adaptation of pancreatic amylase expression is primarily regulated by transcriptional events and α-amylase mRNA levels increase in response to dietary starch (Brannon, 1990). In ruminants, it has been reported to occur at both transcriptional and post-transcriptional levels (Swanson et al., 2000). Because of the difficulties in distinguishing exogenous and endogenous amylase in the intestine, whether the specific regulation in birds was caused directly or indirectly by exogenous amylase and whether it responded at the transcriptional, translational or secretory level remains unclear. It has been reported that pancreatic amylase activity was decreased with a lower starch diet (Xu et al., 2006) and the production of chicken pancreatic amylase is regulated mainly by neural factors instead of gut hormones (Murai et al., 2000). Thus, it is assumed that the decline in α-amylase expression was caused by a breakdown effect of starch in the intestine by exogenous amylase, which might subsequently result in less dietary stimulus to the GIT

neural system and down-regulate the pancreatic amylase expression. Another explanation is based on the conservation model of digestive enzymes which was proposed by Rotheman et al. (2002). Rotheman et al. (2002) pointed out that a large portion of the digestive enzymes secreted by the pancreas are absorbed into blood and recycled in an enteropancreatic circulation similar to enterohepatic circulation. If so, the increased presence of dietary amylase would elevate the amylase level in circulation, which has been proved by Onderci et al. (2006) who found significantly higher amylase activities in serum of broilers supplemented with alpha-amylase-producing bacterial culture, and accordingly increase the conserved amylase in the pancreas by accumulation. As such, we supposed that the increasing involvement of dietary amylase in the enteropancreatic circulation might negatively affect the production of amylase mRNA or protein in the pancreas. The pancreas is responsible for both synthesis and secretion of the digestive enzymes. If the synthetic rate of pancreas equals the rate of enzyme secretion, and the amylase mRNA expression is depressed under the mitigation of neural stimulus or endogenous interference of enteropancreatic circulation, or both, for a long term, the secretion of pancreatic amylase would then be lower and, accordingly, the accumulation of intestinal amylase would be slowed down. This might be helpful to explain the depressed amylase activity in the intestine when a higher amount of dietary amylase was administrated.

In summary, the inclusion of amylase in diets resulted in increased intestinal enzyme activities in young broilers, and benefited digestion and growth in a dose-dependent manner. However, the dose-dependent change patterns of digestive enzymes and growth rate are not consistent. Exogenous enzyme inclusion was also found to affect pancreatic enzyme mRNA levels of broilers in a negative manner, suggesting caution for supplementation of exogenous pancreatic enzyme analogues at a high dose in poultry diets.

REFERENCES

Acamovic, T. 2001. Commercial application of enzyme technology for poultry production. World's Poult. Sci. J. 57:225-243.

Ashild, K. and L. S. Jerry. 1989. Influence of age on lipase, amylase, and protease activities in pancreatic tissue and intestinal contents of young turkeys. Poult. Sci. 68:1561-1568.

Bedford, M. R. and K. Autio. 1996. Microscopic examination of feed and digesta from wheat-fed broiler chickens and its relation to bird performance. Poult. Sci. 75:1-14.

Bedford, M. R. 2000. Exogenous enzymes in monogastric nutrition-their current value and future benefits. Anim. Feed Sci. Technol. 86:1-13.

Bird, F. H. 1971. Distribution of trypsin and α-amylase activities in the duodenum of the domestic fowl. Br. Poult. Sci. 12:373-378.

Brannon, P. M. 1990. Adaptation of the exocrine pancreas to diet. Annu. Rev. Nutr. 10:85-105.

Brock, F. M., C. W. Forsberg and J. C. Buchanan-Smith. 1982. Proteolytic activity of rumen microorganism and effects of proteinase inhibitors. Appl. Environ. Microbiol. 44:561-569.

Cowieson, A. J. 2005. Factors that affect the nutritive value of maize for broilers. Anim. Feed Sci. Technol. 119:293-305.

Cowieson, A. J., T. Acamovic and M. R. Bedford. 2003. Supplementation of diets containing pea meal with exogenous enzymes: Effects on weight gain, feed conversion, nutrient digestibility and gross morphology of the gastrointestinal tract of growing broiler chicks. Br. Poult. Sci. 44:427-437.

Cowieson, A. J., T. Acamovic and M. R. Berford. 2006. Using the precision-feeding bioassay to determine the efficacy of exogenous enzymes- A new perspective. Anim. Feed Sci. Technol. 129:149-158.

Erlanger, B. F., N. Kokowski and W. Cohen. 1961. The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys. 95:271-278.

Glazer, B. and M. L. Steer. 1977. Requirements for activation of trypsinogen and chymotrypsinogen in rabbit pancreatic juice. Anal. Biochem. 77:130-140.

Gracia, M. I., M. J. Aranibar, R. Lazaro, P. Medel and G. G. Mateos. 2003. Alpha–amylase supplementation of broiler diets based on corn. Poult. Sci. 82:436-442.

Iji, P. A., K. Khumalo, S. Slippers and R. M. Gous. 2003. Intestinal function and body growth of broiler chickens fed on diets based on maize dried at different temperatures and supplemented with a microbial enzyme. Reprod. Nutr. Dev. 43:77-90.

Inborr, J. 1990. Enzymes: catalysts for pig performance. Feed Manage. 41:22-30.

Iwamori, M., Y. Iwamori and N. Ito. 1997. Sulfated lipids as inhibitors of pancreatic trypsin and chymotrypsin in epithelium of the mammalian digestive tract. Biochem. Biophys. Res. Commun. 237:262-265.

Kullka, R. and D. Duksin. 1964. Patterns growth and α-amylase activity in the developing chick pancreas. Biochem. Biophys. Acta. 91:506-514.

Lonergan, P., A. Rizos, A. P. Gutierrez, P. M. Moriera, B. Pintado and J. de la Fuente. 2003. Temperal divergence in pattern of messenger RNA expression in bovine embryos cultured from the zyogte to blastocyst stage *in vitro* or *in vivo*. Biol. Reprod. 69:1424-1431.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265-273.

Mahagna, M., I. Nir, M. Larbier and Z. Nitsan. 1995. Effect of age and exogenous amylase and protease on development of the digestive tract, pancreatic enzyme activities and digestibility of nutrients in young meat-type chicks. Reprod. Nutr. Dev. 35:201-212.

Murai, A., S. Satoh, J. Okumura and M. Furuse. 2000. Factors regulating amylase secretion from chicken pancreatic acini *in vitro*. Life Sci. 66:585-591.

Nitsan, Z., G. Avraham, Z. Zoref and I. Nir. 1991. Growth and development of the digestive organs and some enzymes in broiler chicks after hatching. Br. Poult. Sci. 32:515-523.

Nitsan, Z. and Z. Madar. 1978. The level and origin of amylase (EC 3.2.1.1) in the digestive tract of chicks receving trypsin inhibitors in their diet. Br. J. Nutr. 40:235-241.

Noy, Y. and D. Sklan. 1995. Digestion and absorption in the young chick. Poult. Sci. 74:366-373.

Osman, A. M. and N. I. Tanios. 1983. The effect of heat on the intestinal and pancreatic levels of amylase and maltase of laying hens and broilers. Biochem. Physio. 75A:563-565.

Onderci, M., N. Sahin, K. Sahin, G. Gikim, A. Aydin, I. Ozercan and S. Aydin. 2006. Efficacy of supplementation of α-amylase-producing bacterial culture on the performance, nutrient use, and gut morphology of broiler chickens fed a corn-based diet. Poult. Sci. 85:505-510.

Piao, X. S., I. K. Han, J. H. Kim, T. Cho, Y. H. Kim and C. Liang. 1999. Effects of kemzyme, phytase and yeast supplementation on the growth performance and pollution reduction of broiler chicks. Asian-Aust. J. Anim. Sci. 12:36-41.

Rideau, N., Z. Nitzan and P. Mongin. 1983. Activities of amylase, trypsin and lipase in the pancreas and small intestine of the laying hen during egg formation. Br. Poult. Sci. 24:1-8.

Ritz, C. W., R. M. Halet, B. B. Self and D. M. Denbow. 1995. Endogenous amylase levels and response to supplementation feed enzymes in male turkeys from hatch to eight weeks of age. Poult. Sci. 74:1317-1322.

Rothman, S., C. Liebow and L. Isenman. 2002. Conservation of digestive enzymes. Physiol. Rev. 82:1-18.

Sarıçiçek, B. Z., Ü. Kılıç and A. V. Garipoğlu. 2005. Replacing soybean meal (SBM) by canola meal (CM): The effects of multi-enzyme and phytase supplementation on the performance of growing and laying quails. Asian-Aust. J. Anim. Sci 18:1457-1463.

SAS. 1991. SAS User's Guide Version 6.03. SAS Institute, Cary, NC.

Selle, P. H., V. Ravindran, G. Ravindran and W. L. Bryden. 2007. Effects of dietary lysine and microbial phytase on growth performance and nutrient utilisation of broiler chickens. Asian-Aust. J. Anim. Sci. 20:1100-1107.

Somogyi, M. 1960. Modification of two methods for the assay of amylase. Clin. Chem. 6:23-27.

Swanson, K. C., J. C. Matthews, A. D. Matthews, J. A. Matthews, J. A. Howell, C. J. Richards and D. L. Harmon. 2000. Dietary carbohydrate source and energy intake influence the expression of pancreatic α-amylase in lambs. J. Nutr. 130:2157-2165.

Verduin, P. A., J. M. Punt and H. H. Kreutzer. 1973. Studies on the determination of lipase activity. Clinica. Chimica. Acta. 46:11-19.

Xu, M., J. H. Yao, Y. H. Wang and F. N. Wang. 2006. Influence of rumen escape starch on α-amylase activity in pancreatic tissue and small intestinal digesta of lambs. Asian-Aust. J. Anim. Sci. 19:1749-1754.

Wang, Z. R., S. Y. Qiao, W. Q. Lu and D. F. Li. 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestional morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poult. Sci. 84:875-881.

Zanella, I., N. K. Sakomura, F. G. Silversides, A. Fiqueirdo and M. Pack. 1999. Effect of enzyme supplementation of broiler diets based on maize and soybeans. Poult. Sci. 78:561-568.