Asian-Aust. J. Anim. Sci.
Vol. 23, No. 9 : 1198 - 1204
September 2010

www.ajas.info

Effects of Multiple Enzyme (ROVABIO® Max) Containing Carbohydrolases and Phytase on Growth Performance and Intestinal Viscosity in Broiler Chicks Fed Corn-Wheat-Soybean Meal Based Diets

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ABSTRACT : This study was conducted to investigate the effects of dietary supplementation with multiple enzymes composed of phytase plus carbohydrolases (ROVABIO® Max, RM) on growth performance, nutritional availability and intestinal viscosity in broiler chicks. A total of one thousand, one-day-old male broiler chicks were randomly allotted into treatment groups that received one of five experimental diets for 32 days. Each group consisted of 40 birds and all experiments included five replicates. The dietary treatments included PC (a positive control diet), NC1 (65 kcal/kg, 0.15% and 0.10% less ME, available phosphorus and calcium levels, respectively, than the PC diet), NC2 (85 kcal/kg, 0.20% and 0.10% less ME, available phosphorus and calcium levels, respectively, than the PC diet), NC1+RM (NC1 plus ROVABIO® Max) and NC2+RM (NC2 plus ROVABIO® Max). The average body weights, daily body weight gains and feed conversion rates of the chicks fed a diet containing RM improved significantly or tended to improve. The treatments also had no effect on the carcass characteristics or blood parameters, but the viscosity of the intestinal contents of the chicks fed the diet containing RM was significantly lower than that of chicks in the NC without RM groups. Additionally, chicks fed the dietary RM showed increased breaking strength and ash content of the tibia when compared to chicks that received the non-RM diets. Taken together, the results of the present study indicated that the addition of multiple enzymes consisting of phytase plus NSP enzymes improved the growth performance and mineral status of the tibia in broiler chickens fed corn-wheat-soybean meal-based diets with reduced levels of nutrients. Further, these findings suggest that the improved animal performance is associated with reduced intestinal viscosity by the dietary enzyme complex. (**Key Words :** Multiple Enzyme, Growth Performance, Intestinal Viscosity, Tibia Ash, Broiler Chick)

INTRODUCTION

Although feeds that contain high non-starch polysaccharides (NSP) are relatively inexpensive, these products are often not used in broiler feed due to their poor utilization of nutrients and low feed efficiency. It is well known that supplemental enzymes such as β-glucanase, xylanase, protease and amylase break these polymeric chains into smaller pieces, thereby improving their nutritional value (Cowieson and Ravindran, 2008). Additionally, Lazaro et al. (2004) suggested that supplementation of feed with β-glucanase and xylanase improved the average daily gain and feed conversion. They also reported that these enzymes reduced digesta viscosity, which leads to improved contact between endogenous enzymes and nutrients, thereby improving digestibility.

Because phosphorus in the phytate of plant ingredients is not well utilized by chickens, phosphorus is added to diets to meet the requirements for birds. Consequently, phosphorus pollution from poultry production is a major environmental concern. In addition, the use of high levels of phosphorus increases the cost of feed. Several studies have demonstrated that dietary phytase increased the utilization of minerals and energy and nutrient digestibility in wheat based diets (Zyla et al., 2001; Selle et al., 2000). Moreover, supplementation of the diet with phytase was found to lead to a significant increase in the breaking strength and ash content of the tibia of growing birds (Perney et al., 1993).

The synergetic effects of phytase and xylanase on

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Received November 24, 2009; Accepted April 5, 2010

growth performance following simultaneous inclusion in wheat based diets have also been shown in recent studies (Peng et al., 2003; Selle et al., 2003b). For example, xylanase was found to increase the access of phytase to its substrate and facilitate the absorption of nutrients by reducing the intestinal viscosity and releasing encapsulated nutrients (Ravindran et al., 1999; Zyla et al., 1999; Selle et al., 2003b). However, Jacob et al. (2000) reported that dietary supplementation with a combination of pentosanase and phytase did not lead to improved performance. Conversely, Peng et al. (2003) reported that supplementation of the diet with a combination of phytase and xylanase increased phytate digestibility to 77.8% and improved feed efficiency by 7.3%, which was significantly higher than the results obtained when phytase alone was used. Therefore, this study was conducted to investigate the effects of dietary supplementation with a multiple enzyme complex (ROVABIO® Max) of NSP enzymes plus phytase on the growth performance, viscosity of intestinal contents and ash content of the tibia in broiler chickens.

MATERIALS AND METHODS

Enzyme

A multiple enzyme complex was supplied by Adisseo (Adisseo Asia Pacific Pte Ltd., the Adelphi, Singapore). ROVABIO® Max in liquid form is a concentrated solution composed of xylanase (1,100 visco unit/kg), β-glucanase (100 AGL units/kg) and phytase (500 FTU units/kg) that are obtained from two fermentation broths with *Penicillium funiculosum* and *Schizosaccharomyces pombe*.

Animals and experimental design

A total of one thousand, one day old male broiler chicks (Ross 308) were obtained from a commercial hatchery. The birds were individually weighed and then randomly allotted to 25 pens. Each treatment comprised five replications of 40 birds, which were housed in a pen under controlled temperature and humidity. Feed was provided *ad libitum* and the birds had free access to water. The mortalities and health status were observed and recorded daily throughout the entire experimental period. Light was provided 24 h a day and the room temperature was gradually decreased from 33°C at day 1 to 23°C at day 32. The animal care and experimental procedures used in this study conformed to the ethical regulations and guidelines of Konkuk University in Korea. All chicks were fed diets in pelleted form for 32 days. The dietary treatments were as follows: PC (the positive control diet formulated to meet the requirements of all nutrients stated by the NRC), NC1 (65 kcal/kg, 0.15% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet), NC1+RM (NC1 plus ROVABIO® Max), NC2 (85 kcal/kg, 0.20% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet) and NC2+RM (NC2 plus ROVABIO® Max). The dietary levels were formulated according to National Research Council (NRC, 1994) recommendations for broiler chicks and the formula and chemical composition of the experimental diets are shown in Table 1.

Assays

The starter diets were fed for 21 d, after which the birds were provided with finisher diets until the end of the experiment. The body weights and feed intake were recorded on a per pen basis weekly and the feed conversion rates were calculated.

At the end of the experimental period, ten chicks from each treatment group were selected and weighed individually. Blood was drawn from the wing vein using sterilized syringes and then analyzed for various characteristics. Upon necropsy, the abdominal fat, liver, right leg and breast muscle were immediately removed and weighed. After removing the intestinal contents, the empty weights and lengths of the duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum) and the ileum (from Meckel's diverticulum to the ileocecal junction) were measured.

The concentrations of total cholesterol (TC) and the activities of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) in the serum were measured according to the colorimetric method using a cholesterol diagnostic-kit (Cholesterol E kit, Asan Pharmaceutical) and a GOT-GPT assay kit (GOT-GPT assay kit, Asan Pharmaceutical), respectively. The concentrations of blood urea nitrogen and albumin were measured according to the urease-glutamate dehydrogenase kinetic method using pureauto S-UN (DAICHI, Choongwae Pharma Corp.) and the bromcresol green method using clinimate ALB (DAICHI, Choongwae Pharma Corp.) respectively, with a chemistry autoanalyzer (HITACHI 7600-110, Japan).

After measurement of the length and weight of the tibia, the tibia breaking strength was determined using an Instron standard testing machine (Model 4465). For this test, the tibia was placed on two supports (4 cm apart for the tibia) and a perpendicular force (KN) was applied until the tibia fractured. The sheared tibia pieces were collected and defatted, after which the Tibia samples were oven-dried (EYELA NDO-500) at 100°C for 24 h and then weighed to obtain the dry weight. The samples were then ashed in a muffle furnace (Isotemp muffle furnace, Fisher Scientific, Pittsburgh, PA) at 600°C for 24 h in crucibles. Finally, the content of calcium and phosphorus in the tibia was determined using the standard methods (AOAC, 1995).

Table 1. Ingredients and chemical composition of experimental diets[1,2]

Ingredients (%)	Starter (1-21 d)			Finisher (22-32 d)		
	PC	NC1	NC2	PC	NC1	NC2
Yellow corn	47.76	50.78	51.57	52.56	55.61	56.39
Wheat	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal	28.99	28.40	28.22	24.49	23.95	23.82
Corn gluten meal	2.32	2.42	2.47	2.00	2.00	2.00
Meat meal	2.00	2.00	2.00	2.00	2.00	2.00
Tallow	4.67	2.84	2.33	4.80	2.99	2.48
L-lysine HCl (30%)	1.20	1.15	1.14	1.19	1.15	1.14
DL-methionine (99%)	0.28	0.26	0.26	0.25	0.24	0.23
L-threonine (98%)	0.10	0.09	0.08	0.10	0.09	0.08
Salt	0.24	0.23	0.23	0.23	0.23	0.23
Limestone	0.49	0.75	0.93	0.67	0.94	1.11
Dicalcium phosphate	1.47	0.57	0.27	1.21	0.31	0.01
Vit.+Min.mixture [2]	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride (50%)	0.10	0.10	0.10	0.10	0.10	0.10
Salinomycin	0.10	0.10	0.10	0.10	0.10	0.10
Avilamycin	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100
Calculated values						
Crude protein (%)	21.00	21.00	21.00	19.00	19.00	19.00
Crude fat (%)	7.31	5.59	5.10	7.50	5.79	5.31
Crude fiber (%)	2.38	2.40	2.40	2.25	2.27	2.28
Crude ash (%)	5.34	4.72	4.60	5.05	4.43	4.31
Ca (%)	0.80	0.70	0.70	0.80	0.70	0.70
Available P (%)	0.40	0.25	0.20	0.35	0.20	0.15
Lysine (%)	1.26	1.24	1.24	1.14	1.13	1.12
Total TSAA (%)	0.92	0.91	0.90	0.84	0.83	0.82
TMEn (kcal/kg)	3,100	3,035	3,015	3,150	3,085	3,065

[1] PC = A positive control diet formulated to meet the requirement of all nutrients of the NRC; NC1 = 65 kcal/kg, 0.15% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC2 = 85kcal/kg, 0.20% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet.

[2] Vit.+Min.Mineral mixture provides the following nutrients per kg of diet: vitamin A, 12,283 IU; D_3, 3,000 IU; E, 28 ppm; Biotin, 0.27 ppm; Fe, 75 ppm; Zn, 74 ppm; Cu, 74 ppm; Se, 0.31 ppm.

The intestinal contents of the duodenum, jejunum and ileum were collected for determination of the viscosity. To accomplish this, the contents were centrifuged at 9,000 rpm for 10 min at 4°C, after which 0.5 ml of supernatant was placed into a cone and plate viscometer (LVDL-II+P CP, Brookfield, USA). The viscometer was set at 10 rpm for evaluation and the viscosity reading was recorded after 30 s.

The cecal digesta homogenates in saline were serially diluted to 10^6 and the concentration of ammonia was then measured using an ammonia assay kit (Product code AA0100, Sigma) according to the manufacturer's directions. Ammonia reacts with ketoglutaric acid and reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of L-glutamate dehydrogenase to form L-glutamate and oxidized nicotinamide adenine dinucletide phosphate. The decrease in absorbance at 340 nm due to the oxidation of NADPH is proportional to the ammonia concentration.

Statistical analysis

The differences in treatment effects among groups were evaluated by ANOVA using the general linear models procedure of SAS (2002) and significant differences were determined using Duncan's multiple range test at $p<0.05$ (Duncan, 1955). Percentage data were transformed to arc sine percentages before square root percentages ANOVA was performed.

RESULTS AND DISCUSSION

Growth performance

The effects of dietary Rovabio® Max on the growth performance of broiler chickens are shown in Table 2. The final body weight of the chicks fed NC1+RM was highest,

Table 2. Effects of dietary Rovabio® Max on growth performance in broiler chicken[1]

	PC	NC1	NC1+RM	NC2	NC2 +RM
BW (g/bird/d)					
Initial	39.0±0.04	39.0±0.02	39.0±0.03	39.0±0.03	39.0±0.04
Final	1,829.6±29.08^{ab}	1,778.4±12.59^{b}	1,868.2±11.19^{a}	1,620.7±20.18^{c}	1,830.0±25.99^{ab}
BW gain (g/bird/d)					
1-21 d	39.1±0.53^{a}	39.0±0.57^{a}	40.1±0.37^{a}	35.6±0.52^{b}	39.8±0.36^{a}
22-32 d	88.2±2.07^{ab}	83.8±0.54^{b}	89.7±0.52^{a}	75.8±2.01^{c}	86.9±2.08^{ab}
1-32 d	56.0±0.86^{ab}	54.4±0.37^{b}	57.2±0.33^{a}	49.4±0.59^{c}	56.0±0.76^{ab}
Feed intake (g/bird/d)					
1-22 d	59.1±0.83	56.8±1.23	58.5±2.32	58.2±2.37	58.0±1.04
22-32 d	143.1±2.29	140.7±1.12	144.0±0.79	140.8±3.39	140.9±1.04
1-32 d	87.1±0.43	85.4±1.21	86.6±1.61	85.9±1.73	85.9±0.82
FCR (feed/gain)					
1-22 d	1.52±0.03^{ab}	1.46±0.02^{b}	1.46±0.05^{b}	1.63±0.07^{a}	1.46±0.02^{b}
22-32 d	1.62±0.02^{b}	1.68±0.02^{b}	1.61±0.01^{b}	1.87±0.08^{a}	1.63±0.03^{b}
1-32 d	1.56±0.02^{b}	1.57±0.02^{b}	1.51±0.03^{b}	1.74±0.03^{a}	1.53±0.01^{b}

[1] PC = A positive control diet formulated to meet the requirement of all nutrients of the NRC; NC1 = 65 kcal/kg, 0.15% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC1+RM = NC1 plus Rovabio® Max 0.02%; NC2 = 85 kcal/kg, 0.20% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC2+RM = NC2 plus Rovabio® Max 0.02%.

[a-c] Mean±SE values with different superscripts with a row differ significantly ($p<0.05$).

while those of chicks fed diets containing RM were significantly higher than those in the NC1 and NC2 ($p<0.05$). The daily body weight gain during the finisher period (22-32 d) and the entire experimental period (1-32 d) was significantly higher in chicks that were fed diets containing RM than in those that received the NC diets without RM ($p<0.05$). During the starter period (1-21 d), the daily body weight gain of the chicks fed NC1+RM tended to increase when compared to the NC1 group, while that of the NC2+RM was significantly higher than that of the NC2 group ($p<0.05$). The beneficial effects of some feed enzymes on nutrient availability and performance have been established in previous studies. For example, Zanella et al. (1999) demonstrated that dietary enzyme improved energy utilization in corn-soybean diets supplemented with amylase, protease and xylanase. The feed conversion rate of birds that received the NC2+RM diet was significantly improved during the starter, finisher and total periods when compared with that of birds in the NC2 group ($p<0.05$) by 10.5%, 12.9% and 12.1%, respectively. Selle et al. (2003b) reported that supplementation of the diet with phytase plus xylanase increased the growth rate and feed efficiency. In addition, Ravindran et al. (1999) suggested that the addition of phytase plus xylanase to the diet induced similar synergistic responses as lysine, glycine, arginine, methionine, alanine, histidine, leucine, tyrosine and phenylalanine. These results imply that xylanase could increase the access of phytase to phytate in the aleurone layer of wheat, where phytate is concentrated. Therefore, it can be postulated that the addition of Rovabio® Max exerted a synergetic effect that led to increased utilization of available phosphorus, available amino acids and metabolizable energy, which promoted the growth of broiler chicks.

Blood parameters

The dietary effects of Rovabio® Max on various blood parameters in broiler chickens are shown in Table 3. The concentrations of cholesterol, blood urea nitrogen and albumin in serum were not influenced by the dietary treatments. Additionally, there were no significant differences in the activities of serum GOT and GPT among treatment groups. Measurement of GOT and GPT activities are indicative of liver damage in broiler chicks and is therefore a valuable tool for determination of a safe inclusion rate for feed additives. Based on these findings, Rovabio® Max administered at the levels evaluated in this study may not exert adverse effects on broiler chickens.

Carcass characteristics

The dietary effects of Rovabio® Max on the relative weights of the liver, abdominal fat, right leg and right breast muscle in broiler chickens are shown in Table 3. There were no significant differences in the relative weights of the liver, abdominal fat, right leg or right breast muscle among treatment groups. The relative weights of the right leg and right breast muscle in chicks fed diets containing RM were slightly higher than those of chicks in the NC groups without RM, but these differences were not significant. Selle et al. (2003a) found that supplementation of wheat-based diets with xylanase plus phytase increased breast weight by 5.8%, but Zanella et al. (1999) reported that enzyme addition had no effect on the relative weight of leg, breast muscle and wings.

Table 3. Effects of dietary Rovabio® Max on carcass characteristics and blood parameters in broiler chicken[1,2,3]

	PC	NC1	NC1 +RM	NC2	NC2 +RM
Carcass characteristics	---------- g/100 g BW ----------				
Liver	2.47±0.05	2.50±0.08	2.62±0.10	2.49±0.07	2.52±0.06
Abdominal fat	2.31±0.10	2.38±0.07	2.36±0.05	2.30±0.09	2.17±0.09
Breast muscle	8.95±0.13	8.85±0.16	8.91±0.14	8.79±0.12	8.99±0.13
Leg	9.16±0.07	8.86±0.26	9.05±0.17	8.75±0.14	9.27±0.14
Blood parameters					
TC (mg/100 ml)	84.19±6.08	87.39±4.09	87.39±4.26	87.98±3.30	83.33±1.14
GOT (IU/L)	110.56±2.63	111.10±2.82	113.70±2.72	114.43±2.49	112.14±2.24
GPT (IU/L)	6.03±0.49	5.58±0.11	5.96±0.19	5.96±0.23	5.96±0.36
Albumin (g/dl)	1.20±0.04	1.23±0.02	1.27±0.04	1.23±0.02	1.20±0.05
BUN (mg/dl)	2.00±0.15	1.86±0.14	2.12±0.18	1.78±0.09	1.93±0.15

[1] PC = A positive control diet formulated to meet the requirement of all nutrients of the NRC; NC1 = 65 kcal/kg, 0.15% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC1+RM = NC1 plus Rovabio® Max 0.02%; NC2 = 85 kcal/kg, 0.20% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC2+RM = NC2 plus Rovabio® Max 0.02%.

[2] TC = Total cholesterol; GOT = Glutamic-oxaloacetic transaminase; GPT = Glutamic-pyruvic transaminase; BUN = Blood urea nitrogen.

[3] Values are presented Mean±SE.

Table 4. Effects of dietary Rovabio® Max on weight and length of small intestine in broiler chicken[1,2,3]

	PC	NC1	NC1+RM	NC2	NC2+RM
Weight	---------- g/100 g BW ----------				
DU	0.55±0.02	0.48±0.02	0.49±0.02	0.50±0.03	0.47±0.02
JE	0.97±0.06	0.82±0.05	0.93±0.04	0.86±0.04	0.88±0.04
IL	0.72±0.05	0.60±0.04	0.63±0.03	0.62±0.03	0.64±0.02
Length	---------- cm/100 g BW ----------				
DU	1.64±0.05	1.57±0.05	1.57±0.04	1.67±0.04	1.54±0.04
JE	3.80±0.11	3.94±0.12	3.77±0.09	4.01±0.12	3.72±0.10
IL	4.12±0.10	4.02±0.10	3.97±0.12	4.40±0.12	4.01±0.09

[1] PC = A positive control diet formulated to meet the requirement of all nutrients of the NRC; NC1 = 65 kcal/kg, 0.15% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC1+RM = NC1 plus Rovabio® Max 0.02%; NC2, 85 kcal/kg, 0.20% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC2+RM = NC2 plus Rovabio® Max 0.02%.

[2] DU = duodenum; JE = jejunum; IL = ileum.

[3] Values are presented Mean±SE.

The dietary effects of Rovabio® Max on the relative weight and length of the small intestine in broiler chickens are shown in Table 4. The treatments had no effect on the relative weight and length of the small intestine. Wu et al. (2004) suggested that individual addition of phytase and xylanase led to a significant reduction in the relative length and weight of the small intestine ($p<0.05$). However, Brenes et al. (1993) demonstrated that supplementation of wheat-based diets with xylanase had no effect on the relative weight of the small intestine, pancreas, proventriculus and liver in broiler chicks.

Tibia properties

The dietary effects of Rovabio® Max on various tibia properties in broiler chickens are shown in Table 5. There were no significant differences in the relative weight and length of the tibia among treatments. The tibia breaking strength and ash were significantly higher in chicks fed diets containing RM than in those of the NC groups that did not contain RM ($p<0.05$). The improvement of ash percentage may have been related to increases in the phosphorus and calcium retentions from the phytate-mineral complex by the action of the phytase. Several authors have reported similar results and these findings are considered to be a good indication of the association of increased tibia mineralization with the addition of phytase to the diet (Ahmad et al., 2000; Watson et al., 2006).

Viscosity of intestinal contents

The intestinal contents are known to increase in the presence of NSP, resulting in negative effect on performance. However, NSP enzymes counteract these adverse effects. The effects of dietary Rovabio® Max on the viscosity of intestinal contents are presented in Table 6. The viscosity of the intestinal contents of chicks fed diets containing RM was significantly decreased than that of chicks in the NC groups without RM ($p<0.05$). Similar results have been reported by Zanella et al. (1999) in response to a corn-soybean meal diet with a mixture of amylase, protease and xylanase. Additionally, Wu et al.

Table 5. Effects of dietary of Rovabio® Max on tibia properties in broiler chicken[1]

	PC	NC1	NC1+RM	NC2	NC2+RM
Tibia weight (g/100 BW)	0.85±0.02	0.84±0.02	0.86±0.02	0.88±0.02	0.87±0.02
Tibia length (cm)	9.84±0.08	9.70±0.03	9.75±0.06	9.64±0.06	9.75±0.05
Breaking strength (KN)	0.42±0.02^{ab}	0.37±0.02^{bc}	0.45±0.03^{a}	0.31±0.02^{c}	0.42±0.02^{ab}
Ash (%)	42.30±0.72^{a}	39.08±0.58^{b}	42.29±0.69^{a}	35.63±1.30^{c}	41.25±0.54^{ab}
Calcium (% of ash)	35.54±0.27^{a}	35.24±0.26^{ab}	36.53±0.40^{a}	33.33±0.42^{c}	34.04±0.71^{bc}
Phosphorus (% of ash)	17.15±0.12^{a}	16.34±0.19^{b}	16.64±0.18^{ab}	14.91±0.36^{c}	14.97±0.25^{c}

[1] PC = A positive control diet formulated to meet the requirement of all nutrients of the NRC; NC1 = 65 kcal/kg, 0.15% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC1+RM = NC1 plus Rovabio® Max 0.02%; NC2 = 85 kcal/kg, 0.20% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC2+RM = NC2 plus Rovabio® Max 0.02%.

[a-c] Mean±SE values with different superscripts with a row differ significantly ($p<0.05$).

Table 6. Effects of dietary Rovabio® Max on viscosity of intestinal contents and cecal ammonia concentration in broiler chicken[1]

	PC	NC1	NC1+RM	NC2	NC2+RM
Viscosity (mPas)	4.97±0.12^{bc}	5.24±0.21^{ab}	4.59±0.17^{c}	5.55±0.14^{a}	4.62±0.15^{c}
Ammonia concentration (μg/ml)	1.87±0.03	1.90±0.04	1.78±0.08	1.90±0.08	1.77±0.06

[1] PC = A positive control diet formulated to meet the requirement of all nutrients of the NRC; NC1 = 65 kcal/kg, 0.15% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC1+RM = NC1 plus Rovabio® Max 0.02%; NC2, 85 kcal/kg, 0.20% and 0.10% point less ME, available phosphorus and calcium levels than the PC diet; NC2+RM = NC2 plus Rovabio® Max 0.02%.

[a-c] Mean±SE values with different superscripts with a row differ significantly ($p<0.05$).

(2004) demonstrated that the viscosity of digesta from the duodenum of broiler chicks decreased ($p<0.05$) in response to supplementation of the diet with xylanase and phytase. These results are in agreement with those of a study conducted by Steenfeldt and Pettersson (2001). In the present study, dietary supplementation of NSP enzymes plus phytase reduced the viscosity of intestinal contents by 12.4 and 16.8%, respectively. These results suggest that NSP enzymes able to break down the cell wall matrix may facilitate the release of nutrients encapsulated in cell walls, lowering the viscosity of the intestinal contents, resulting in an easier access of phytase.

Cecal ammonia concentration

The presence of ammonia in chicken barns can have an adverse effect on the performance and health of both the farmed animals and their human attendants. The effects of dietary Rovabio® Max on cecal ammonia concentration in broiler chickens are shown in Table 6. Chicks that received diets containing RM tended to have a lower cecal ammonia concentration. Liu et al. (2007) reported that the addition of xylanase to wheat-based diets reduced the cecal ammonia concentration to 35.4%. Therefore, the use of RM may exert beneficial effects on the environment of poultry houses.

In conclusion, recent studies have shown that the simultaneous inclusion of phytase and NSP enzymes in diets is advantageous (Selle et al., 2003b; Wu et al., 2004) and the present data confirms these findings. Improved performance in response to treatment with NSP enzymes plus phytase was generally associated with reduced viscosity of intestinal contents and increased tibia mineralization.

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