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EFFECT OF DIETARY PROBIOTICS SUPPLEMENTATION WITH DIFFERENT NUTRIENT DENSITY ON GROWTH PERFORMANCE, NUTRIENT RETENTION AND DIGESTIVE ENZYME ACTIVITIES IN BROILERS

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ABSTRACT

This study was involving a 2×2 factorial arrangement (2 nutrient density diets with 2 levels of probiotics), to evaluate effect of probiotics on growth performance, nutrient retention and digestive enzyme activities of broilers.Three hundred and twenty one-day-old chickens were randomly divided into four groups with 10 replicate of 8 broilers each for a 42-d feeding trial.The dietary were formulated: a positive control diet (PC) and a negative control diet (NC) to contain 320 KJ metabolizable energy /kg diet and 0.85 percentage crude protein less than PC, which both were supplemented with0 or 100 mg/kg probiotics. Broilers fed NC diet tended to reduce body weight（BW and average daily gain (ADG) (P=0.063) in starter phase (1-21 d). Probiotics treatments improved 21-d BW, ADG (P<0.05) and feed/gain ratio (P=0.078) in starter phase. Probiotics improved the retention of CP (P<0.05) during 11-14 d, but reduced the activity of lipase in pancreas of broilers (P<0.01) at 21 d. It was concluded that low nutrient density tended to reduce BW and ADG of broilers in starter phase, but probiotics improved the starter phase of growth performance and retention of CP, reduced pancreatic lipase activity of broilers.

Key words: probiotic; nutrient level; nutrient retention; digestive enzyme; broiler.

INTRODUCTION

Feedstuffs shortage and environmental pollution had received a worldwide attention in animal production. The contribution of low-nutrient diets supplemented with enzymes or probiotics products can be considerable, which could improve feed conversion ratio (F/G) and nutrient retention. Zanella *et al.* (1999) demonstrated that supplementation of exogenous enzymes (xylanase, protease and amylase) allowed performance to be maintained on a maize-soybean meal diet with low nutrient level. The energy and amino acid digestibility of the diets for broilers was also improved by 3 %. It was also demonstrated that enzyme supplementation improved energy digestibility in diets with lower levels of apparent metabolizable energy (AME) (Kocher *et al.*, 2003; Zhou *et al.*, 2009). Probiotics are live microorganisms that exert health effects beyond inherent basic nutrition (Fuller, 1989). In poultry industry, probiotics applications has widely been shown to improve the barrier function of intestine and reduce pathogenic problems in gastrointestinal tract thus leading to the enhancement of immune response and replacement of sub-therapeutic antibiotics (Rial *et al.*, 2000; Soderholm *et al.*, 2001; Ouwehand *et al.*, 2002; Medici *et al.*, 2004; Galdeano and Perdigón, 2006). Some studies had shown that probiotics supplementation in chicken diets improved digestion (Verschuere *et al.*, 2000), nutrient retention and F/G (Palliyaguru *et al.*, 2004). Jin *et al.* (2000) reported that inclusion of a probiotic resulted in significantly higher amylase enzyme activities in the small intestine of broilers. Furthermore, probiotics, particularly members of the genus *Bacillus* secrete a wide range of enzymes (Pugsley and Schwartz, 1985). Recently, most studies on probiotics supplemented to adequate nutrient diet had been carried out on gastrointestinal microflora and immunity of animals (Elmer *et al.*, 1996; Prioult *et al.*, 2003; Galdeano *et al.*, 2007), however, data of nutritional effects of probiotics in a broiler diet with lower nutrient level are relatively lacking. Therefore, the objective of this experiment was to examine the effect of probiotics supplementation on growth performance, nutrient retention and digestive enzyme activities of broilers fed diets with different nutrient density.

MATERIALS AND METHODS

Probiotics preparation: A commercial probiotics provided by Beijing E-feed& E-vet Co., Ltd. (Beijing, China), contained *Bacillus subtilis*, *Lactobacillus acidophilus* and *Bacillus licheniformis*, with a total concentration of 1 $\times 10^{10}$ cells per gram. The original mixed culture broth (7 strains of *Bacillus subtilis*, 3 strains of *Lactobacillus acidophilus* and 2 strains of *Bacillus licheniformis*) was spray dried and mixed with cornstarch and skimmed milk powder to yield a final product. The pH and temperature of culture was 5.5-6.5 and 37°C, respectively. The amount of bacterial inoculum

was 3 % (vol. /vol.). The product was stored at room temperature.

Diets and experimental design: The animal management and sampling procedures were in accordance with the guidelines of Nanjing Agricultural University Institutional Animal Care and Use Committee.

Two basal diets i.e. negative control diet (NC) and positive control diet (PC) with different energy and crude protein (CP) concentrations were formulated according to the experimental design. The NC diet had approximately 320 KJ AME /kg diet and 0.85% CP less than PC diet.

Four diets i.e. NC diet without probiotic, PC diet without probiotic, NC diet with 100 mg/kg probiotic and PC diet with 100 mg/kg probiotic were offered to the respective groups of birds. The basal diets without antibiotics were commercial maize-soybean meal diets of Nanjing Kangxin Poultry Co. Ltd. (Nanjing P.R. China).The formulation and nutrient level of basal diets are shown in Table 1.

Three hundred and twenty one-day-old healthy Arbor Acres chickens were obtained from a commercial hatchery (Hewei, Anhui province, P. R. China) and completely randomized divided into four groups with 10 replicate cages of 4 males and 4 females each for a 42-d feeding trial. The chicks were raised in cages and all birds were placed in a room maintained at 32-34 °C for the first week and then reduced by 2-3 °C per week.The lighting schedule provided 24 h of light per day throughout the experiment. The chicks were allowed *ad libitum* access to feed and water via trough.

Sample collection procedures: Eight broilers with 8 replicates per treatment were randomly selected at 21 and 42 d after feed with drawl, and killed by cervical dislocation. Pancreas was quickly excised, and the contents taken from the small intestine were from the distal end of the duodenum to the jejunal-ileo junction. To ease the sampling process, the mesentery was cut to uncoil the tract. A homogenous intestinal digesta sample was collected by massaging the tract from both ends. Care was taken to remove excess tissue from each digesta sample to minimize interference with the enzyme assay. The intestinal samples and pancreas were stored immediately at −70 °C until used.

Growth performance: The body weight (BW), feed consumption and mortality of the chicks were recorded. The average BW of chicks was determined at 21 and 42 d. Feed was withdrawn for 12 h, with water being provided *ad libitum*, before the chicks were weighed at 21dand 42 d. Feed intake was measured during the 42-d experiment, and the feed/gain ratio (F/G) was calculated. The average BW, average daily gain (ADG), average daily feed intake (ADFI) and F/G ratio were used to determine the growth performance of the chicks.

Apparent retention of nutrients: At 11 d and 35 d of age, six chickens per treatment (one chicken per replicate) were randomly selected and allocated into metabolism cages (40 cm × 40 cm × 35 cm). After 16 h of adaptation to experimental diets, the birds were fed their respective experimental diet for a 3-d period followed by a 72-h complete excreta collection, and allowed *ad libitum* access to water, then reintroduced into the growth performance. Excreta were collected twice per day from a plastic tray placed under the cages, stored at -20 °C, and finally pooled for each cage. Feathers and shredded dry skin were removed carefully before excreta were stored in sealed plastic bags and preserved immediately in freezer (−20 °C). The excreta samples were then dried for 48 h in an oven at 65 °C. The dried excreta were allowed to equilibrate to atmospheric conditions for 24 h before being weighed. Feed and excreta samples were then ground through a 0.45-mm screen. The samples were analysed (AOAC, 1990) for dry matter (DM, 934.01), crude protein (CP, 976.05), ether extract after HCl treatment (EE, 920.39). Gross energy (GE) was determined using an adiabatic calorimeter (SXHW-III, Tianyu, Hebi, China). The apparent retention of nutrients was then calculated according to Adeola*et al.* (2008).

Enzyme activity assay: For enzymatic analysis, the pancreas and digesta samples were homogenised(1:9, wt/vol) for 60 s with ice-cold 154 mmol/L sodium chloride solution, and then centrifuged at 4,550 ×g for 15 min at 4°C. The supernatants from both intestinal and pancreas samples were divided into small portions and stored at −70°C for enzyme assays. Amylase activity was determined using the iodometric method of Somogyi (1960) and one amylase unit is defined as the amount of enzyme that hydrolyses 10 mg of starch in 30 min. Lipase activity was determined using the turbidimetric method of Verduin *et al.* (1973) and one lipase unit is defined as the amount of enzyme that hydrolyses 1 μmol of olive oil per min. Trypsin activity was determined as described by Schwert and Takenaka (1955) using N-benzoyl-L-arginine ethyl ester (BAEE) as substrate, and the absorbance of the product was measured at 253 nm. One unit of trypsin was defined as the amount of enzyme that increases 0.003 of absorbance of product per minute. Pancreatic protein concentration was determined according to the method of Bradford (1976), using bovine serum albumin as a standard protein. Enzyme activity was expressed as units per gram of digesta for intestinal samples and units per milligram of protein for pancreatic samples.

Statistical Analysis: Data obtained were analyzed by General Linear Model (GLM) procedure of SPSS (2008). Two-way ANOVA was employed to determine the main effects (nutrient density, probiotics) and their interactions by using GLM procedure of SPSS .The differences were

considered to be significant at $P< 0.05$. *P* values between 0.05 and 0.10 were considered as a trend.

RESULTS AND DISCUSSION

The growth and feed efficiency of chickens during the growing phase may be affected by the nutrient levels of diets (Pestia and Smitha, 1983). This study showed that broilers fed the NC diet tended to reduce 21-d BW (P=0.063) and ADG (P=0.063) in the starter phase (1-21 d) (Table 2),because growth performance of broiler was dependent on the concentration of metabolizable energy and dietary protein, and decreasing either dietary protein or energy content significantly decreased BW (Pestia and Smitha, 1984). It has been suggested that probiotics supplementation can improve broiler growth (Zulkifli*et al.*, 2000; Willis *et al.*, 2007). In the present trial, probiotics supplementation in both diets increased 21-d BW and ADG (P<0.05), the same trend was observed for F/G (P=0.078) (Table 2). There was no effect of probiotics on ADFI, suggesting that the observed performance responses were due to changes in apparent retention of energy and CP rather than increased nutrient intake. Furthermore, probiotics addition to low-nutrient diet conferred to similar performance to the birds that received PC diet, indicating that this probiotics may compensate for the reduction in performance brought about by feeding the NC, but the exact deficiency of the NC is not clear. However, there was no reduction in performance of broilers during the finisher phase as a result of probiotics or nutrient density treatment (Table 2).It may be so that even the NC met the nutrient requirements of the birds over the entire 42d period sufficiently well that no further improvement in performance was noted with increased energy and protein density. As a result it is not clear if the probiotics could prove useful to 42d of age by improving nutrient retention or not in diets limiting in nutrient density.

The apparent retention of DM, organic matter (OM), CP, EE and GE were unaffected by nutrient density treatments (Table 3 and Table 4). Probiotics supplementation increased the apparent retention of CP (P<0.05) during 11-14 d (Table 3), but had no effect on the apparent retention of GE, DM, OM and EE during 11-14 d or 35-38 d (Table 3 and Table 4). The apparent retention of CP (11-14 d) was improved by probiotics, a finding that is consistent with data of Palliyaguru*et al.* (2004), who observed improvements in CP retention of broilers on the starter phase of growth when supplementing the diet with 0.02% probiotics containing *Lactobacillus*, *Streptococcus* and *Bifidobacterium*. This effect may be mediated by an improvement in the main functions of digestion, absorption and propulsion in the gastrointestinal tract (Fioramonti *et al.*, 2003). These findings suggested that part of the beneficial effects of probiotics on early phase of growth performance may be mediated via improvement in the apparent retention of CP, reducing nitrogen excretion.

The enzyme activities in intestine, such as amylase, trypsin and lipase, play an important role in the digestion of nutrients and ultimately, in poultry performance and nutrient retention (Bird, 1971; Zafrira *et al.*, 1991). In this study, the intestinal enzyme activities were unaffected by neither probiotics nor nutrient density treatment and there was no interaction (Table 5). Some studies have shown that probiotics supplementation improved the enzyme activities in intestine of broilers (Wang and Gu, 2010). The results in the present study demonstrated that this probioticshad no effect on the intestinal enzyme activities of amylase, lipase and trypsin, similar to the finding of Bedford and Schulze (1998), who reported that the enzymes produced by probiotic would represent, at most, only a small contribution to the total enzyme activity of the intestine. However, the present study showed that probiotics supplementation decreased 21-d lipase activity in pancreas of broilers (P<0.01) (Table 6). It can be partly explained that exogenous enzyme addition to broiler had negatively affected pancreatic enzyme production and decreased mRNA expression (Jiang *et al*. 2008), and probiotics, especially members of the genus *Bacillus* secreted a wide range of enzymes (Pugsley and Schwartz, 1985; Kato *et al.* 2010). These results indicated that the probiotics secrete lipase and thus the requirements of the pancreas are reduced ,or pancreatic lipase activity levels were reduced but intestinal levels of lipase and indeed fat retention remained constant suggests that either the longevity of the lipase in the intestine is increased with probiotics, or both. Meanwhile, probiotics supplementation did not affect the activities of endogenous amylase and trypsin (Table 6), finding that was not in agreement with report of Wang *et al.* (2010), who observed that feeding of broilers with *B. coagulans* NJ0516 significantly increased protease and amylase activities compared with control group. It indicated that different probiotic strains had different capacity to produce enzymes and/or stimulate the endogenous enzyme production of broilers, and these changes suggested that probiotics may affect endogenous enzyme activities by different mechanisms in pancreas.

Table 1. Ingredient composition, calculated and analyzed nutrient content of the basal NC and PC diets (g/kg, as-fed basis unless otherwise stated)

Item	1-21 d		22-42 d	
Ingredient	PC	NC	PC	NC
Maize	576	574	625	633
Soybean meal	324	319.5	263	249
Corn gluten meal	30	20	35	30
Soybean oil	28.4	25.6	37	30.8
Dicalcium phosphate	17.5	17.5	16.5	16.5
Limestone	12.8	12.8	12	12
L-Lysine	1.9	1.9	2.2	2.2
DL-Methionine	1.4	1.4	1.2	1.2
Salt	3	3	3	3
Premix [1]	5	5	5	5
Carrier(zeolite)	0	19.3	0.1	17.3
Total	1,000	1,000	1,000	1,000
Calculated nutrient values[2]				
Metabolizable energy (MJ/kg)	12.51	12.29	13.00	12.68
Crude protein	211.4	202.9	192.8	184.3
Calcium	9.7	9.7	9.0	9.0
Available phosphorus	4.2	4.2	4.0	4.0
Lysine	11.1	10.8	10.0	9.6
Methionine	4.9	4.7	4.5	4.4
Methionine + cysteine	8.5	8.2	7.8	7.5

[1] Premix provided per kilogram of diet: transretinyl acetate, 10,000 IU; cholecalciferol, 3,000 IU; all-rac-α-tocopherol acetate, 20 mg; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 400 mg; calcium pantothenate, 10 mg; pyridoxine·HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; cobalamin, 0.013 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulfate), 8 mg; Mn (from manganese sulfate), 110 mg; Zn (from zinc oxide), 65 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg. [2]The nutrient levels were as fed basis. PC = Positive control; NC = Negative control

Table 2.Effects of probiotics on growth performance of broilers fed diets with different nutrient levels

Treatment[1,2,3]	1-21 d				22-42 d				1-42 d		
	21-d BW(g)	ADG (g/d)	AFDI (g/d)	FCR	42-d BW(g)	ADG (g/d)	AFDI (g/d)	FCR	ADG (g/d)	AFDI (g/d)	FCR
PC	700	31.1	46.1	1.48	1,998	61.8	129.2	2.09	46.5	86.7	1.87
NC	696	30.9	46.2	1.49	1,972	60.6	127.7	2.11	45.8	87.0	1.90
PC+ probiotics	748	33.4	46.7	1.40	2,034	61.2	131.4	2.15	47.3	87.9	1.86
NC+ probiotics	703	31.3	46.1	1.48	1,995	61.9	125.4	2.03	46.0	84.6	1.84
S.E.M.	6.17	0.29	0.12	0.01	11.7	0.49	0.965	0.02	0.229	0.47	0.01
Main effects											
Probiotics treatment											
No probiotics	698[a]	31.0[a]	46.2	1.49	1,985	61.2	128.5	2.10	46.3	86.5	1.88
Probiotics	726[b]	32.3[b]	46.4	1.44	2,014	61.7	128.4	2.09	46.6	86.2	1.85
Dietary treatment											
PC	724	32.3	46.4	1.44	2,016	61.5	130.3	2.12	46.9	87.3	1.86
NC	700	31.1	46.2	1.48	1,983	61.3	126.6	2.07	46.1	85.4	1.87
P value											
Probiotics	0.040	0.040	0.887	0.078	0.225	0.731	0.975	0.721	0.513	0.805	0.389
Diet	0.063	0.063	0.764	0.118	0.186	0.790	0.068	0.216	0.101	0.053	0.606
Probiotics× Diet	0.109	0.109	0.375	0.220	0.776	0.378	0.259	0.100	0.238	0.164	0.738

[1]Values within a column not sharing the same superscript are different at $P<0.05$; n = 10.
[2]PC = Positive control; NC = Negative control; S.E.M. = Standard error of means.
[3]BW = Body weight; ADG = Average daily gain; ADFI = Average daily feed intake; FCR = Feed conversion ratio.

Table 3.Effects of probiotics on nutrient retention (11-14 d) of broilers fed diets with different nutrient levels

Treatment[1,2]	11-14 d nutrient retention				
	Dry matter	Organic matter	Crude protein	Ether extract	Gross energy
PC	0.71	0.73	0.56	0.71	0.74
NC	0.69	0.71	0.53	0.69	0.73
PC + probiotics	0.70	0.73	0.58	0.74	0.75
NC+ probiotics	0.70	0.73	0.58	0.70	0.74
S.E.M.	0.004	0.003	0.007	0.012	0.003
Main effects					
Probiotics treatment					
No probiotics	0.70	0.72	0.55 [a]	0.70	0.74
Probiotics	0.70	0.73	0.58 [b]	0.72	0.75
Dietary treatment					
PC	0.71	0.73	0.57	0.73	0.75
NC	0.70	0.72	0.56	0.70	0.74
P value					
Probiotics	0.378	0.269	0.036	0.462	0.150
Diet	0.176	0.095	0.178	0.304	0.193
Probiotics× Diet	0.134	0.164	0.299	0.659	0.369

[1]Values within a column not sharing the same superscript are different at $P<0.05$; n = 6.
[2]PC = Positive control; NC = Negative control; S.E.M. = Standard error of means.

Table 4. Effects of probiotics on nutrient retention (35-38 d) of broilers fed diets with different nutrient levels.

Treatment[1,2]	35-38 d nutrient retention				
	Dry matter	Organic matter	Crude protein	Ether extract	Gross energy
PC	0.67	0.65	0.45	0.56	0.70
NC	0.66	0.63	0.47	0.57	0.68
PC + probiotics	0.70	0.68	0.53	0.65	0.73
NC+ probiotics	0.68	0.65	0.49	0.57	0.69
S.E.M.	0.008	0.009	0.014	0.023	0.009
Main effects					
Probiotics treatment					
No probiotics	0.67	0.64	0.46	0.57	0.69
Probiotics	0.69	0.67	0.51	0.61	0.71
Dietary treatment					
PC	0.69	0.67	0.49	0.61	0.72
NC	0.67	0.64	0.48	0.57	0.69
P value					
Probiotics	0.212	0.171	0.113	0.386	0.240
Diet	0.326	0.206	0.764	0.475	0.136
Probiotics× Diet	0.710	0.600	0.278	0.311	0.525

[1]Values within a column not sharing the same superscript are different at $P<0.05$; n = 6.
[2]PC = Positive control; NC = Negative control; S.E.M. = Standard error of means.

Table 5. Effects of probiotics on intestinal enzyme activities of broilers fed diets with different nutrient levels.

Treatment[1,2]	21 d (U/g digesta)			42 d (U/g digesta)		
	Amylase	Lipase	Trypsin	Amylase	Lipase	Trypsin
PC	1,908	1.44	94,084	1,326	5.55	105,190
NC	1,355	0.98	87,762	1,289	4.83	105,120
PC + probiotics	1,963	1.65	82,632	1,135	6.92	102,180
NC+ probiotics	1,601	1.43	86,639	1,365	6.11	104,980
S.E.M.	173	0.17	8,285	116	0.37	5,196
Main effects						
Probiotics treatment						

No probiotics	1,631	1.21	90,923	1,308	5.19	105,155
Probiotics	1,782	1.54	84,636	1,251	6.52	103,580
Dietary treatment						
PC	1,935	1.54	88,358	1,231	6.24	103,685
NC	1,478	1.20	87,200	1,327	5.47	105,050
P value						
Probiotics	0.671	0.335	0.707	0.808	0.096	0.881
Diet	0.209	0.323	0.945	0.684	0.320	0.897
Probiotics× Diet	0.786	0.722	0.758	0.574	0.954	0.892

[1]Values within a column not sharing the same superscript are different at $P<0.05$; n = 8.
[2]PC = Positive control; NC = Negative control; S.E.M. = Standard error of means.

Table 6. Effects of probiotics on pancreatic enzyme activities of broilers fed diets with different nutrient levels.

Treatment[1,2]	**21 d (U/mg protein)**			**42 d (U/mg protein)**		
	Amylase	**Lipase**	**Trypsin**	**Amylase**	**Lipase**	**Trypsin**
PC	128	1.27	11,254	102	0.86	10,005
NC	106	1.42	10,971	115	1.12	8,844
PC + probiotics	127	0.94	12,671	95	0.83	10,701
NC+ probiotics	115	0.77	15,469	82	0.87	10,804
S.E.M.[3]	10	0.07	1,025	9	0.05	629
Main effects						
Probiotics treatment						
No probiotics	117	1.35[A]	11,113	109	0.99	9,425
Probiotics	121	0.86[B]	14,070	89	0.85	10,753
Dietary treatment						
PC	128	1.11	11,963	99	0.85	10,353
NC	111	1.10	13,220	99	1.00	9,824
P value						
Probiotics	0.844	0.003	0.168	0.291	0.220	0.307
Diet	0.430	0.939	0.548	0.993	0.190	0.680
Probiotics× Diet	0.810	0.259	0.463	0.501	0.312	0.622

[1]Values within a column not sharing the same superscript are different at $P<0.05$ or $P<0.01$; n = 8.
[2]PC = Positivecontrol;NC = Negative control; S.E.M. = Standard error of means.

Conclusion: It was concluded that the diet with low nutrient level tended to reduce 21-d BW and ADG of broilers in starter phase. Probiotics supplementation improved performance and apparent retention of CP, reduced pancreatic lipase activity of broilers fed a maize-soybean meal diet with low nutrient level in the starter phase.

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