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A note on the effects of a combination of an enzyme complex and probiotic in the diet on performance of broiler chickens

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The object of this experiment was to investigate the effect of an enzyme complex and a probiotic mixture, offered either singly or in combination, in the diet of broiler chickens (from 1 to 47 days of age) on growth and digestive tract weight and length. A total of 480 1-day old male Ross-308 broilers were allocated to 6 treatments, with 4 replicates of 20 birds each. The treatments were three concentrations of an enzyme complex (0, 250 or 500 mg/kg of the diet) each with and without probiotic supplementation. Inclusion of the enzyme complex linearly improved body weight (BW) and food conversion ratio (FCR) and reduced the relative weight (g/g carcass weight) of duodenum and the length of the jejunum. Probiotic inclusion only improved FCR. However, there was an interaction between the linear effect of enzyme concentration and response to probiotic inclusion for BW, FCR and the relative weight of the duodenum. It is concluded that the combination of the enzyme complex and probiotic can improve the performance more than either supplement used on its own.

Keywords: broiler; enzyme; performance; probiotic

Introduction

Previous studies have indicated a beneficial effect of dietary inclusion of probiotics (Mountzouris *et al*. 2010), enzymes (García *et al*. 2008) or enzyme complex (Oryschak *et al*. 2010; Rebolé *et al*. 2010) on the performance of broilers. An improvement in food efficiency due to dietary inclusion of probiotic has been attributed changes in the gastrointestinal tract that facilitate

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digestion and absorption of nutrients. This may reflect various physiological changes in the contents of gastrointestinal tract, including increased short chain fatty acid production, antimicrobial substances, stimulation of the immune system, and competition for nutrients and adhesion sites in the gastrointestinal tract (Jin *et al*. 1997; Vandeplas *et al*. 2009; Taheri *et al*. 2009), and therefore may contribute towards excluding pathogen colonization in the host.

Exogenous enzymes are usually incorporated in poultry wheat- or barley-based diets to degrade the anti-nutritive, factors such as arabinoxylans of wheat, or β-glucans of barley, and consequently improve nutrient digestibility and the growth performance of poultry (Simon 2000; García *et al*. 2008). Several studies have shown that such enzymes also reduce the colonization of the gut by pathogens (Vahjen *et al*. 1998; Danicke *et al*. 1999; Hubener, Vahjen and Simon 2002). Hence, it was supposed that there may be an interaction between the effects of probiotic and enzyme complex especially in a diets based on wheat and barley. For example, Vandeplas *et al*. (2009) investigated the efficiency of a *Lactobacillus plantarum*-xylanase combination on growth performance of broilers infected with *Salmonella* serotype Typhimurium. Therefore the objective was to evaluate the interaction effect between an enzyme complex and probiotic inclusion for growth performance of non-infected broiler chickens. The commercially suggested level of an enzyme complex and also half concentration with or without probiotic supplementation were used to test this assumption.

Material and Methods

Four hundred and eighty 1-day old male Ross-308 broiler chicks were randomly divided into 24 groups. Each treatment had 4 replicates and each replicate was assigned to a cage (2.5 m × 1.25 m) with 20 broilers. Birds were raised in on-floor cages and in an environmentally controlled house; they were exposed to a 23:1 light:dark cycle and had *ad libitum* access to water and feed. The experiment consisted of a 3×2 factorial arrangement of treatments: three concentrations of enzyme complex (0, 250 and 500 mg/kg of the diet) with and without probiotic supplementation. The commercially recommended levels of the probiotic supplement (Primalac, Star-Labs, USA) were used; 900, 450 and 225 mg/kg of the diet for starter, grower and finisher periods, respectively. The probiotic mixture contained *L. acidophilus*, *L. casei*, *Bifidobacterium bifidum*, *Enterococcus faecium* at viable number of 2×10^8 colony forming units per 1 kg of the supplement. Commercially recommended inclusion of the enzyme complex was 500 mg/kg of the diet; the complex contained β-glucanase, α-amylase, cellulase, protease and lipase. All procedures were approved by the Animal Care and Welfare Committee of the University of Tehran. All diets were based on wheat-barley-soya. Addition of bran, corn, gluten meal and vegetable oil were chosen so that all diets had a metabolizable energy concentration of 12.1 MJ/kg and 221.5, 184.1 and 172.3 g/kg crude protein for starter (1 to 10 days), grower (11 to 28 days), and finisher (29 to 47 days) periods, respectively. The formulation of the diets was based on guidelines for Ross-308 broilers (Broiler Nutrition Specification 2007).

Broilers were weighed on days 10, 28 and 47 to determine average body weight (BW) per cage and weight gain was calculated. Average initial weight of all chicks was 39 g. Food intake per cage was recorded at the same ages and food conversion ratio (FCR) was calculated for all growth periods. At 47 days of age, 2 birds from each cage were sacrificed to measure length and relative weight (g/g of carcass weight) of duodenum, jejunum and ileum.

Data were analyzed as a completely randomized design using Proc GLM procedure of SAS (2003) with pen as the experimental unit. The response to increasing concentration of dietary enzyme and its interaction with probiotic were partitioned using orthogonal polynomial contrasts. Since the quadratic components were not significant for any response variable, these are not displayed in the results section. Means were compared using the Tukey-Kramer procedure.

Results and Discussion

Performance improvement of broiler chickens by addition of feed additives in the diets has been known for several decades. Enzymes in wheat or barley-based diet and probiotic as an alternative of antibiotics, are some of the outstanding ones for this purpose. Combination of some feed additives with together in the diet formulation sometime can enhance their effectiveness. The results (Table 1) show that inclusion of the enzyme complex linearly improved BW at 28 ($P < 0.003$) and 47 ($P < 0.002$) days of age, representing increases of 8.4% compared to the non-supplemented group, but addition of probiotic had no effect on growth at any point. Food intake was not affected by the treatment, but addition of the enzyme complex improved the FCR in a linear fashion ($P < 0.01$). The high concentration of the enzyme complex reduced FCR ($P < 0.01$) by 0.13 over the 47 day period. Furthermore, addition of the probiotic improved FCR ($P < 0.05$) (for days 1 to 10 and 1 to 47). There was an interaction between the linear response to enzyme complex concentration and probiotic inclusion for growth performance. Probiotic inclusion boosted the effect of enzyme complex on BW (at 28 and 47 days of age) and on FCR (for the whole period). Thus the combination of enzyme and probiotic in the diet can enhance the food efficiency more than that when used singly. One reason for this interaction may be a reduction of intestinal pathogen level: enzymes, by degrading the anti-nutritive factors and so reducing the viscosity of the digesta, and probiotic, by increasing the beneficial microflora, production of antimicrobial components, short chain fatty acids and lowering the pH of digestive tract.

The inclusion of the enzyme complex caused a linear reduction in the length of the jejunum ($P < 0.01$) and in the relative weight of the duodenum ($P < 0.01$) and ileum ($P < 0.05$) and this effect exhibited a significant interaction with probiotic inclusion for each of these components (Table 2). The interaction reflected the fact that the linear reduction was greater in the absence of probiotic than when probiotic was included. The positive effect of enzyme complex on length of jejunum and relative weight of duodenum implies an effect on degradation of anti-nutritive factors. Such degradation reduces the viscosity of the digesta and consequently a negative effect on intestinal weight and length. Typical probiotics do not have any ability to hydrolyze non-starch polysaccharides. Probiotic inclusion is not expected to change the physical properties of the digestive tract; the interaction of enzyme level and probiotic inclusion for the relative weight of duodenum was unexpected and we cannot offer any explanation.

Table 1. Growth performance of birds offered diets containing an enzyme complex with or without probiotic

Item	Level†	Body weight (g) at day			Food intake (g) for days				Food conversion ratio for days			
		10	28	47	1 to 10	11 to 28	29 to 47	1 to 47	1 to 10	11 to 28	29 to 47	1 to 47
Probiotic	0	179	914	2179	226	1154	2555	3892	1.63[a]	1.59	2.02	1.84[a]
	1	186	930	2197	219	1113	2490	3781	1.49[b]	1.51	1.97	1.77[b]
Enzyme complex	0	177	881[b]	2113[b]	224	1131	2543	3861	1.63	1.61	2.06	1.87[a]
	1	183	918[ab]	2150[b]	217	1127	2458	3761	1.52	1.55	2.00	1.80[ab]
	2	188	966[a]	2301[a]	225	1142	2568	3887	1.52	1.48	1.92	1.74[b]
s.e. for												
Probiotic		3.4	13.9	23.7	2.3	20.7	41.5	47.4	0.045	0.031	0.039	0.023
Enzyme complex		4.2	17.1	29.0	2.9	25.4	50.8	58.1	0.056	0.038	0.048	0.028
Significance of:												
Linear effect of enzyme level			**	**						*		**
Interaction‡			**	**						*		**

†For probiotic 0 = without and 1= with probiotic; for enzyme complex 0 = none, 1 = 250 mg/kg of the diet, and 2 = 500 mg/kg of the diet.
‡Between the linear effect of enzyme level and probiotic inclusion; subclass means for BW at day 28 were: 864,903, 975,899,934 957 (s.e. 24.1) g for increasing levels of enzyme complex in the absence and presence of probiotic, respectively; the corresponding means for body weight at day 47 were: 2098, 2172, 2266, 2128, 2128, 2336 (s.e. 41.1); and 1.89,1.84,1.80,1.86,1.76,1.69 (s.e. 0.041) for food conversion ratio for the interval 1 to 47 days.
abcMeans for probiotic inclusion or enzyme level, within a column, without a superscript in common are significantly different ($P < 0.05$).

Table 2. Mean values for relative weight and the length of the components of the small intestine of birds offered diets containing combinations of an enzyme complex with or without probiotic

Item	Digestive tract measurement†					
	DR	JR	IR	DL	JL	IL
Enzyme complex (mg/kg diet)						
0	0.93[a]	1.97	1.69	32.0	83.6[a]	85.6
250	0.86[ab]	1.87	1.66	30.2	77.6[ab]	79.4
500	0.79[b]	1.77	1.52	32.1	76.0[b]	78.0
s.e.	0.029	0.072	0.053	0.78	2.01	2.47
Significance of :						
Linear effect of enzyme level	**		*		**	*
Interaction‡	**		*		*	

† DR, JR and IR = relative weights (g/g carcass) of duodenum, jejunum and ileum, respectively; DL, JL and IL= length (cm) of duodenum, jejunum and ileum, respectively.

‡Between linear effect of enzyme level and probiotic inclusion; the subclass means for DR were: 0.98, 0.89, 0.75, 0.87, 0.84, 0.84 (s.e. 0.041) g/g for increasing levels of enzyme complex in the absence and presence of probiotic, respectively; the corresponding means for IR were: 1.81, 1.75, 1.45, 1.58, 1.57, 1.59 (s.e. 0.076), respectively; and for JL the corresponding means were: 82.6, 80.5, 76.6, 84.3, 78.3, 79.3 (s.e. 3.50), respectively.

[abc]Means, within a column, without a superscript in common are significantly different ($P < 0.05$).

It is concluded that the combination of the enzyme complex and probiotic can improve the performance more than either supplement used on its own.

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