# An investigation of the effects of using an enzyme-probiotic combination on broilers performance

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***Abstract:***

**BACKGROUND:** Growth promoters are chemical and biological substances that are added to livestock food with the aim to improve the growth of chickens in fattening and the utilization of food, and in this way achieve better production and financial results. **OBJECTIVES:** An experiment was conducted to evaluate the effects of enzyme complex, probiotic, and their combination on performance of broilers fed a basal wheat-barley-soybean meal diet from 1 to 47 d of age. **METHODS:** A total of 480-day-old male broiler chicks (Arbor Acres) were randomly assigned to 6 treatments, with 4 replicate cages per treatment and 20 birds per cage. The experiment consisted of a 3×2 factorial arrangement of the treatments, with 3 concentrations of enzyme complex (0, half of the commercial suggested level or commercial suggested level) and 2 concentrations of probiotic (0 and suggested level). **RESULTS:** Results showed that the suggested level of enzyme complex could improve body weight and feed conversion ratio ($p<0.05$), but addition of probiotic only decreased the feed conversion ratio ($p<0.05$). There was no interaction between enzyme complex and probiotic on performance ($p>0.05$). Probiotic supplementation did not improve the efficacy of enzyme complex at any levels. The examination of length and relative weight of different regions of intestine showed that only enzyme complex could decrease the relative weight of duodenum and length of jejunum; however, there was no interaction between treatments for these parameters. **CONCLUSIONS:** This research did not demonstrate any interaction effect between enzyme complex and probiotic on broilers performance fed wheat-barley-soybean meal diet.

## Introduction

Research focusing on bird's endogenous enzymes (Krogdahl et al., 1989; Sklan, 2002) suggested that the young bird might be limited in the types and amounts of enzymes necessary to utilize carbohydrate and protein in diet at early age; thus, affecting nutrient digestibility (Noy and Sklan, 1994). Probiotics and exogenous enzymes have been used commercially for a number of years to improve poultry nutrient digestibility. There are many documents about the beneficial effects of probiotics (Patterson and Burkholder, 2003; Awad et al., 2009; Peric et al., 2009) and enzymes (Wang et al., 2005; Peric et al., 2008) in broiler nutrition. Using probiotics in poultry diets has a beneficial effect on broiler performance (Khaksefidi and Ghoorchi, 2006), modulation of intestinal microflora and pathogen inhibition (Mountzouris et al., 2007), immunomodulation (Haghighi et al., 2005), and certain haematobiochemical parameters (Mathivanan et al., 2007). On the other hand, 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). It was shown that diet supplementation with probiotic and/or such enzymes decrease the most intestinal pathogens (Fukata et al., 1991; La Ragione et al., 2004; Kizerwetter-Swida and Binek, 2005). It was supposed that a relationship between the effects of probiotic and enzyme complex especially in a wheat-barley based diet could be found. Vandeplas, et al. (2009) investigated the efficiency of a L. plantarum-xylanase combination on growth performance of broilers infected with *S. Typhimurium*. Also, Rebole et al. (2010) examined the effects of inulin (as a prebiotic) and enzyme complex, individually or in combination, in broiler nutrition. The objective of the present study was to evaluate the effects of enzyme complex, probiotic, and their combination on performance of broilers fed a basal wheat-barley-soybean meal diet.

## Materials and Methods

**Birds, housing and management:** A total of four hundred and eighty 1-d-old Arbor Acres broiler chicks (average 41±0.3 g body weight) were randomly assigned to 6 treatments of 4 floor pen replicates. The birds were housed in deep litter pens and reared from day one to seven weeks. The temperature of the experimental room was maintained at 32±2 °C during the first week of trial and then reduced by 2 °C each week till it reached 24 °C, which was maintained for the rest of the period. All treatments were given ad libitum access to water and feed.

**Experimental diets:** The experiment consisted of a 3×2 factorial arrangement of the treatments with 3 concentrations of enzyme complex (0, half of the commercial suggested level, and commercial suggested level) and 2 concentrations of probiotic (0 and suggested level). Suggested levels of probiotic were 900, 450, and 225 mg/kg of the diet for starter, grower, and finisher periods, respectively. The probiotic (PrimaLac®) contained the viable microorganisms of $2\times10^8$ cfu (of *L. acidophilus*, *L. Casei*, *B. bifidum*, *E. faecium*). Suggested levels of enzyme complex (Combo®) containing β-glucanase, α-amylase, cellulase, protease, and lipase were 500 mg/kg of the diet. The basal diet was a mash wheat-barley-soybean meal that was formulated according to the Arbor Acres broiler nutrient requirements for starter (1 to 10 d), grower (11 to 28 d), and finisher (29 to 47 d) periods. Compositions of the basal diet and calculated nutrient levels for the experiment are presented in Table 1.

**Data collection:** Chickens were weighed on 10, 28, and 47 d to determine average body weight (BW). Feed intake (FI) per cage was recorded on the same dates and feed conversion ratio (FCR) was calculated for all periods. At 47 d of age, two birds of each cage were killed to measure the relative weight of duodenum, jejunum, and ileum (g/g of carcass weight) and their length as well.

**Statistical analysis:** Data were analyzed as a completely randomized design using the ANOVA procedures of Statistical Analysis System soft ware (SAS, 2005) and means were compared using Duncan's multiple range test. A single cage represented the experimental unit (replicate) for all measured parameters. The results were presented as means and SEM calculated by standard procedures. Differences among treatments were considered significant when $p< 0.05$. The model is:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$$

Where Yijkln is the observed response, μ is the overall mean, $A_i$ is the effect of enzyme complex, $B_j$ is the effect of probiotic, $(AB)_{ij}$ is the interaction between the effect of two factors, and $e_{ijk}$ is the remained effects.

## Results

Results showed that inclusion of a single enzyme complex or probiotic in the wheat-barley-soybean meal diet can improve performance. Addition of suggested level of enzyme complex in the diet could increase the BW by 9.4, 8.4, and 8.4% on grower, finisher, and whole period, respectively; however, supplementation of probiotic did not have any effect on this parameter (Table 2). FI was not affected by treatments, while enzyme complex supplementation decreased the FCR of whole period by 0.13 and probiotic by 0.14 and 0.08 for starter and whole periods, respectively (Table 2). Half of the suggested level of enzyme complex did not display any beneficial effect on performance and also there was

Table 1. Formulation of the diet and estimated chemical composition of experimental rations. 1supplied per kilogram diet: vitamin A= 9000 IU , vitamin D3= 2000 IU, vitamin B1= 18 IU, vitamin B3= 9.8 IU, vitamin B6= 2.9 IU, vitamin B12= 0.15 IU, vitamin E= 18 IU, vitamin K3= 2mg, vitamin B2=6.6 mg, vitamin B5 = 29.7 mg, vitamin B9= 1 mg, vitamin H2= 0.1 mg, Cholin chloride= 500 mg , Mn= 99.2 mg , Zn= 84.7 mg , Fe = 5mg , Co= 1mg, Se=0.2 mg, I= 0.992 mg.

| Experiment Period / Ingredients | Starter (1-10 Days) | Grower (11-28 Days) | Finisher (29-47 Days) |
|---|---|---|---|
| Wheat | 300 | 300 | 300 |
| Barley | 300 | 300 | 300 |
| Soy bean meal | 170.7 | 200 | 175 |
| Corn gluten meal | 145.8 | 69.4 | 0 |
| Wheat bran | 0 | 30 | 100 |
| Oil | 32.5 | 60 | 90 |
| Di calcium phosphate | 20.8 | 16 | 13 |
| Calcium carbonate | 17.3 | 14 | 14 |
| Methionine | 0.6 | 0.7 | 0.1 |
| Lysine | 4.4 | 2.1 | 0.2 |
| Salt | 2.9 | 2.8 | 2.7 |
| Premix1 | 5 | 5 | 5 |
| Calculated Analysis | | | |
| M.E.(Kcal.Kg) | 2838 | 2902 | 2948 |
| C.P. | 22.1 | 16.47 | 15.84 |
| Lysine | 1.38 | 1.09 | 0.86 |
| Methionine | 0.539 | 0.33 | 0.24 |
| Methionine + Cystine | 0.920 | 0.58 | 0.48 |
| Calcium | 1.00 | 1.55 | 1.72 |
| P (available) | 0.50 | 0.49 | 0.48 |

Table 2. Effects of enzyme complex, probiotic, or their combination on growth performance. [(a-b)] Means within a row lacking a common superscript are significantly different (*). [(1)] Concentrations of enzyme complex are 1 (0), 2 (half of the suggested level) and 3 (suggested level). [(2)] Concentrations of probiotic are 1 (0) and 2 (suggested level). [(3)] BWG=Body Weight Gain. [(4)] FI=Feed Intake. [(5)] FCR=Feed Conversion Ratio.

| Treatments | | BWG[(3)] (gr) | | | FI[(4)] (gr) | | | | FCR[(5)] (gr/gr) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Enzyme[(1)] (%) | Probiotic[(2)] (%) | 10 days | 28 days | 47 days | 1-10 days | 11-28 days | 29-47 days | 1-47 days | 1-10 days | 11-28 days | 29-47 days | 1-47 days |
| 0 | 0 | 177 | 881[(ab)] | 2113[(b)] | 224 | 1131 | 2543 | 3861 | 1.63 | 1.61 | 2.06 | 1.87[(a)] |
| 50 | 0 | 183 | 918[(ab)] | 2150[(b)] | 217 | 1127 | 2458 | 3761 | 1.52 | 1.55 | 2.00 | 1.80[(ab)] |
| 100 | 0 | 188 | 966[(a)] | 2301[(a)] | 225 | 1142 | 2568 | 3887 | 1.52 | 1.48 | 1.92 | 1.74[(b)] |
| SEM | 4.2 | 17.1 | 29.0 | 2.9 | 25.4 | 50.8 | 58.1 | 0.056 | 0.038 | 0.048 | 0.028 | |
| 0 | 0 | 179 | 914 | 2179 | 226 | 1154 | 2555 | 3892 | 1.63[(a)] | 1.59 | 2.02 | 1.84[(a)] |
| 0 | 100 | 186 | 930 | 2197 | 219 | 1113 | 2490 | 3781 | 1.49[(b)] | 1.51 | 1.97 | 1.77[(b)] |
| SEM | | 3.4 | 13.9 | 23.7 | 2.3 | 20.7 | 41.5 | 47.4 | 0.045 | 0.031 | 0.039 | 0.023 |
| 0 | 0 | 174 | 864 | 2098 | 229 | 1123 | 2550 | 3883 | 1.70 | 1.64 | 2.06 | 1.89 |
| 0 | 100 | 180 | 899 | 2128 | 219 | 1140 | 2535 | 3839 | 1.56 | 1.58 | 2.07 | 1.86 |
| 50 | 0 | 174 | 903 | 2172 | 221 | 1160 | 2537 | 3845 | 1.66 | 1.61 | 2.00 | 1.84 |
| 50 | 100 | 193 | 934 | 2128 | 214 | 1095 | 2379 | 3677 | 1.39 | 1.49 | 1.99 | 1.76 |
| 100 | 0 | 190 | 975 | 2266 | 228 | 1180 | 2579 | 3948 | 1.52 | 1.52 | 2.00 | 1.80 |
| 100 | 100 | 185 | 957 | 2336 | 223 | 1104 | 2556 | 3826 | 1.52 | 1.45 | 1.85 | 1.69 |
| SEM | | 6.0 | 24.1 | 41.1 | 4.1 | 36.0 | 71.9 | 82.2 | 0.079 | 0.054 | 0.068 | 0.04 |

not any interaction between enzyme complex and probiotic. The means of the length and the relative weight of duodenum, jejunum, and ileum for dietary treatments are presented in Table 3. The suggested level of enzyme complex only could decrease ($p<0.05$) the relative weight of duodenum and length of jejunum, in comparison with the control group. Probiotic supplemented group did not show significant difference, and there was no interaction between treatments for these parameters.

## Discussion

Beneficial microflora promote gut health by influencing enterocyte turnover; competing with pathogenic bacteria for nutrients and binding sites,

Table 3. The ratio of intestine regions weight/carcass weight and their length in birds, fed diet contained enzyme complex, probiotic, or their combination. [(a-b)] Means within a row lacking a common superscript are significantly different (*). [(1)] DR=Relative Weight of Duodenum; JR= Relative Weight of Jejunum; IR= Relative Weight of Ileum; DL=Duodenum Length; JL=Jejunum Length; IL=Ileum Length, Relative weight was based on g of each section of intestine/g of total weight of carcass, and the unit of length of each section of intestine was Cm. [(2)] Concentrations of enzyme complex are 1 (0), 2 (half of the suggested level) and 3 (suggested level). [(3)] Concentrations of probiotic are 1 (0) and 2 (suggested level).

| **Treatments** | | **Items[(1)]** | | | | | |
|---|---|---|---|---|---|---|---|
| Enzyme[(2)] (%) | Probiotic[(3)] (%) | DR | JR | IR | DL | JL | IL |
| 0 | 0 | 0.93[(a)] | 1.97 | 1.69 | 32.0 | 83.6[(a)] | 85.6 |
| 50 | 0 | 0.86[(ab)] | 1.87 | 1.66 | 30.2 | 77.6[(ab)] | 79.4 |
| 100 | 0 | 0.79[(b)] | 1.77 | 1.52 | 32.1 | 76.0[(b)] | 78.0 |
| SEM | | 0.029 | 0.072 | 0.053 | 0.78 | 2.01 | 2.47 |
| 0 | 0 | 0.87 | 1.82 | 1.67 | 31.5 | 78.8 | 81.3 |
| 0 | 100 | 0.85 | 1.92 | 1.58 | 31.4 | 79.3 | 80.7 |
| SEM | | 0.023 | 0.059 | 0.043 | 0.63 | 1.64 | 2.02 |
| 0 | 0 | 0.98 | 2.02 | 1.81 | 31.7 | 82.6 | 86.8 |
| 0 | 100 | 0.87 | 1.91 | 1.58 | 32.3 | 84.6 | 84.3 |
| 50 | 0 | 0.89 | 1.79 | 1.75 | 31.3 | 79.7 | 80.5 |
| 50 | 100 | 0.84 | 1.95 | 1.57 | 29.1 | 75.6 | 78.3 |
| 100 | 0 | 0.75 | 1.65 | 1.45 | 31.6 | 74.1 | 76.6 |
| 100 | 100 | 0.84 | 1.89 | 1.59 | 32.7 | 77.8 | 79.3 |
| SEM | | 0.041 | 0.103 | 0.076 | 1.103 | 2.847 | 3.504 |

and producing bacteriostatic compounds that limit the growth of pathogenic bacteria (Farthing, 2004). They also can improve the digestion and absorption of nutrients and immune function (Mountzouris et al., 2010).

The antinutritional effect of wheat arabinoxylans and β-glucans of barley has been shown to be correlated mainly to the entrapment of nutrients in the polysaccharides structure, the so-called cage effect (Simon, 2000) and to the increased viscosity of the intestinal content in the lumen, in relation with increased bacterial populations of the gut (Mathlouthi et al., 2002; Choct et al., 2004). Exogenous enzymes are usually incorporated in poultry wheat- or barley-based diets to degrade the antinutritional arabinoxylans of wheat or β-glucans of barley, which may consequently improve the nutrient digestibility and the growth performance of poultry (Simon, 2000; García et al., 2008). Several studies have shown that such enzymes have also been found to reduce the bacterial colonization in the gut (Vahjen et al., 1998; Danicke et al., 1999; Hubener et al., 2002).

Although only two birds from each cage were killed at the end of the experiment to measure the relative weight of duodenum, jejunum and ileum and their length, the results showed that the positive effect of enzyme complex on length of jejunum and relative weight of duodenum implies its degradation activity against antinutrients, and therefore it can be more useful than probiotic for reduction of adverse effects of antinutrients on intestinal thickness and weight.

Although enzyme complex and probiotic supplementation could enhance the feed efficiency separately, the probiotic does not have any beneficial impacts on the effectiveness of enzyme complex. This evidence shows that probiotic could not make a condition for better activity of enzyme complex or vice versa.

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# بررسی اثرات استفاده توام آنزیم-پروبیوتیک بر عملکرد جوجه های گوشتی

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## چکیده

**زمینه مطالعه:** محرک های رشد موادشیمیایی وبیولوژیکی هستندکه باهدف بهبودرشدجوجه‌ها وبهبوداستفاده ازمواد غذایی به جیره اضافه شده و بدین گونه باعث تولید بیشتر وسود بالاتر می شوند. **هدف:** این تحقیق به منظور بررسی اثرات استفاده ازآنزیم، پروبیوتیک و ترکیب آنها برروی عملکرد جوجه‌های گوشتی با جیره غذایی بر پایه گندم، جو، سویا ازسن ۱ تا ۴۷ روزگی انجام شد. **روش کار:** ۴۸۰ جوجه گوشتی نر (نژاد آربورآکرز) به طور تصادفی به ۶ تیمار، با ۴ تکرار برای هر تیمار و ۲۰ پرنده در هر قفس تقسیم شدند. این آزمایش شامل آرایش فاکتوریل ۲ × ۳ بود، در این آزمایش ۳ غلظت ازآنزیم (صفر، نصف مقدار تجاری و مقدار تجاری) و ۲ غلظت از پروبیوتیک (صفر و مقدار تجاری) در گروه‌های مختلف استفاده شد. **نتایج:** نتایج نشان داد که سطح پیشنهادی آنزیم می تواند باعث بهبود وزن و ضریب تبدیل غذایی شود ($p<0/05$)، اما اضافه کردن پروبیوتیک به تنهایی باعث کاهش ضریب تبدیل شد ($p<0/05$). اثر متقابلی بین آنزیم و پروبیوتیک برروی عملکرد جوجه‌ها دیده نشد ($p>0/05$). پروبیوتیک باعث بهبود عملکرد آنزیم در سطوح مختلف نشد. آزمایش طول و وزن نسبی مناطق مختلف روده نشان داد که تنها آنزیم می تواند وزن نسبی و طول ژژنوم و اثنی عشر را کاهش دهد. **نتیجه‌گیری نهایی:** این بررسی هیچ اثر متقابلی بین آنزیم و پروبیوتیک بر عملکرد جوجه‌های گوشتی تغذیه شده با جیره پایه گندم، جو، سویا نشان نداد.

**واژه‌های کلیدی:** جوجه گوشتی، آنزیم، عملکرد، پروبیوتیک

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