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## Multi-Enzyme Supplementation to Peak Producing Hens Fed Corn-Soybean Meal Based Diets

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Abstract: This experiment was conducted to evaluate the effects of multi-enzyme supplementation on laying performance, metabolic profile and egg quality of peak producing hens. Lohman layers (n = 144) were blocked according to the location of cages. After one week of the adaptation period, hens were randomly assigned to receive one of three corn-soybean meal based diets supplemented with multi-enzyme (0, 1, or 2 g/kg) from 30 to 46 weeks of age as 12 replicate cages of 4 hens. The active ingredients of the multienzyme supplement were fungal xylanase, fungal  $\beta$ -gluconase,  $\alpha$ -amylase, pectinase,  $\beta$ -gluconase, endo- $\beta$ gluconase, pentosonase, pectinase and hemicellulase. Egg production (EP) and feed intake (FI) were measured daily and egg weight was measured fortnightly. Feed conversion ratio (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced. Two eggs were collected randomly from each cage every 4 weeks to determine egg quality. Body weights (BW) were measured at the beginning and end of the experiment. Blood was also sampled at the end of the experiment to evaluate metabolic profile. The data were analyzed using ANOVA as repeated measures with time being as subplot. The multi-enzyme supplementation did not affect BW, FI and EP; decreased FI; and improved FCR. Except for serum albumin and yolk index, none of metabolic profile and egg quality parameters was affected by the dietary treatments. In conclusion, despite no changes in egg production, decreased feed intake and consequently, improved feed conversion in response to multi-enzyme supplementation could be attributed to enhanced utilization of nutrients.

Key words: Multi-enzyme, corn-soybean meal based diet, laying performance, metabolic profile, egg quality

#### Introduction

In poultry operations, feed cost has always been one of the major issues. Enzyme supplementation as a feed additive has become common since last four decades (Chesson, 1993; Jensen et al., 1957). Moreover, their usage is especially common in European countries due to primarily their positive effects on animal performance as well as their lacking harmful effects on consumers (Dierck, 1989). Enzymes are a group of protein molecules with unique ability to catalyze biochemical reactions. Cereals are major constituents of poultry diets. Replacement of barley and oats with corn reduces feed cost in especially broiler operations. However, the presence of undegradable and complex carbohydrates and the low actual energy utilization limits their widespread use in broiler feeds. They are known as non-starch polysaccharides (NPS) and exert antinutritional properties (Nunes and Malmlof, 1992; Rainbird et al., 1984). Thus, enzymes are supplemented to improve nutritive value through hydrolyzing the mixedlinked  $\beta$ -glucan of endosperm walls of barley and oats and arabinoxylan of endosperm wall of rye (Broz and

Frigg, 1986; Elwinger and Saterby, 1987). Chickens are not capable of hydrolyzing NPS's that mask protein and carbohydrate (Pettersson and Åman, 1989). Non-starch polysaccharides cause an increase in viscosity of digesta and in intestinal tension, which impair diffusions of nutrients and reduce availability of nutrients for digestion and absorption due to sticky droppings and consequently, result in decreased energy value of feedstuff and worsened feed conversion (Annison, 1992; Bedford and Classed, 1992; Choct and Annison, 1992a). Thus, destruction of gel-forming NPS leached from cereal wall resulting from enzyme supplementation is of great importance for poultry species that have a short passage rate (Yu et al., 1998). Because of the presence of soluble glucan and arabinoxylan in endosperm wall, corn and wheat are unresponsive to enzyme supplementation (Veldman and Vahl, 1994).

Legume seeds also contain NPS's such as hemicelluloses, mannan, stachyose and raffinose,  $\alpha$ -galactosides of sucrose up to 27% of total NPS (Chesson, 1987; Irish  $et\ al.$ , 1995). Chickens have no galactosidase activity, thus corn-soybean based diets

Table 1: Ingredients of the experimental diets

0	1	2
491.0	491.0	491.0
222.5	222.5	222.5
50.0	50.0	50.0
40.0	40.0	40.0
60.0	60.0	60.0
20.0	20.0	20.0
22.0	22.0	22.0
2.5	2.5	2.5
	1.0	2.0
86.0	86.0	86.0
4.0	4.0	4.0
1.0	1.0	1.0
1.0	1.0	1.0
	suppleme 0 491.0 222.5 50.0 40.0 60.0 20.0 22.0 2.5 86.0 4.0 1.0	491.0         491.0           222.5         222.5           50.0         50.0           40.0         40.0           60.0         60.0           20.0         20.0           22.0         22.0           2.5         2.5            1.0           86.0         86.0           4.0         4.0           1.0         1.0

<sup>1</sup>Active component was fungal xylanase, fungal β-gluconase, α-amylase, pectinase, β-gluconase, endo-β-gluconase, pentosonase, pectinase and hemicellulase. <sup>2</sup>Per kg contains: Vitamin A, 15.000 IU; cholecalciferol, 1500 ICU, vitamin E (dl-α-tocopheryl acetate), 30 IU; menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; panthotenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B<sub>12</sub>, 15 mcg; Mn, 80.0 mg; Zn, 60.0 mg; Fe, 30.0 mg; Cu, 5.0 mg; I, 2.0 mg; and Se, 0.15 mg.

without enzyme such supplementation, as αgalactosidases, xylanase and pectinases (arabinogalactinase, rhamnogalacturonase galactonase) may result in excessive gas production and diarrhea. However, removal of these compounds up to 90% or supplementation of  $\alpha$ -galactosidase with and without invertase failed to improve apparent metabolizable energy content of soybean meal (Irish et al., 1995). In laying hens fed barley-based diets, supplementation of  $\beta$ -gluconase alone failed to improve laying performance, but not of multi-enzyme containing cellulase, protease and  $\beta$ -glucanase (Chesson, 1993). Numerous studies have been conducted to determine the effect of enzymes on availability of nutrients and performance of broiler fed diets containing cereal grains that are rich in NPS. Few studies involving supplementing diets with enzymes in laying hens are available. Therefore, the aim of this study was to investigate the effects of multi-enzyme supplementation on laying performance, metabolic profile and egg quality of peak-producing hens fed corn-soybean meal based diet.

#### **Materials and Methods**

Animals, diets, and management: The Research Animal Ethic Committee of Atatürk University approved this experimental protocol. One hundred and forty four Lohman layers, weighing 1.53 kg at age of 30 weeks with uniformity of 92% (the number of hens weighing between 0.9-1.1% of the mean BW) were selected from the University Research Farm. Hens were blocked according to the location of cages (48x45x45 cm, width

x depth x height). After one week of the adaptation period, hens were assigned randomly to receive corn-soybean diets containing 0, 1, or 2 gram per kilogram enzyme supplement (Farmazyme  $2010^{\text{TM}}$ , Farmavet, Istanbul, Turkey). Each experimental group was replicated in 12 cages, each containing 4 hens.

The experimental diets (Table 1) were formulated to be isolocaloric and isonitrogenous and met the NRC nutrient recommendations (NRC, 1994). The enzyme supplement contained fungal xylanase, fungal  $\beta$ -gluconase,  $\alpha$ -amylase, pectinase,  $\beta$ -gluconase, endo- $\beta$ -gluconase, pentosonase, pectinase and hemicellulase and it was added into the experimental diets at the expense of wheat bran. The diets contained an average of 89.8% dry matter and 2787 kcal/kg metabolizable energy, 17.9% crude protein, 3.3% crude fiber, 12.6% ash, 3.46% Ca and 0.61% P.

During the experimental period (from week 30 to 46), hens were fed *ad libitum* once daily at 08:30 hours and water was available via nipples all the times. Hens were exposed to a 17:7 light:dark cycle.

Sample collection and analytical procedure: Feed samples collected monthly were analyzed for dry matter, crude protein, crude fiber and ash contents according to procedures outlined by Association of Official Analytical Chemists (AOAC, 1990). Metabolizable energy, Ca and P contents of the experimental diets were calculated from tabular values of feedstuffs for chickens reported by Jurgens (1996). Feed intake and egg production were measured daily. Two eggs were collected bi-weekly from each cage. After storing for 24 hours at room temperature, they were weighed. Body weights (BW) were measured at the beginning and the end of the experiment. Feed conversion efficiency was expressed as kilogram feed intake per kiligram egg production.

About 3 ml of blood samples were collected from two hens in each cage into additive-free vacutainers at the end of the experimental period for metabolic profile. Serum was obtained following centrifugation at 3000 g for 15 min at 20°C. Aliquots were kept at -20°C until laboratory analyses for concentrations of glucose, triglyceride, cholesterol, very low-density lipoprotein, total protein and albumin using commercial kits (DDS®, Diasis Diagnostic Systems Co., Istanbul, Turkey).

To assess egg quality parameters, two eggs were randomly collected from each cage every 4 weeks. Egg quality parameters were calculated using following formulas and methods as summarized by Ergün *et al.* (1987): shape index (%) = (egg width, cm/egg length, cm)\*100; shell strength (kg cm<sup>-2</sup>) was determined by using machine with the spiral pressure system, shell thickness (mm 10<sup>-2</sup>) was determined in 3 different parts by using micrometer; albumen index (%) = (albumen height, mm/average of albumen length, mm and

Table 2: The effects of enzyme supplementation on laying performance of hens during the peak period

Parameter	Level of	enzyme¹, g/kg			Contrasts², F	) <	
	0	1	2	SEM	C vs. E	 L	Q
Initial BW, kg	1.54	1.52	1.52	0.02	0.28	0.40	0.51
Final BW, kg	1.61	1.59	1.59	0.03	0.48	0.56	0.72
BW change, %3	-4.57	-4.80	-4.52	1.76	0.96	0.99	0.91
Egg yield, %4	88.0	88.9	88.9	0.9	0.41	0.48	0.68
Feed intake, g4	131.5	122.9	125.6	1.7	0.001	0.02	0.007
Egg weight, g⁴	60.3	60.1	60.5	0.3	0.94	0.70	0.41
FCR <sup>4,5</sup>	2.50	2.34	2.36	0.04	0.007	0.03	0.1

<sup>1</sup>Least square means. <sup>2</sup>C vs. E = control vs. multi-enzyme supplementation; L = linear effect of multi-enzyme supplementation; Q: quadratic effect of multi-enzyme supplementation. <sup>3</sup>BW change = (initial BW-final BW)\*100/initial BW. <sup>4</sup>Time effect (P < 0.0001). <sup>5</sup>FCR = feed conversion ratio (feed intake:egg yield:, kg:kg).

Table 3: The effects of enzyme supplementation on metabolic profiles of hens during the peak period

Parameter	Level of enz	:yme <sup>1</sup> , g/kg		SEM	Contrasts², F		
	0	1	2		C vs. E	L	Q
Amylase, U/L	340.0	430.0	364.0	42.4	0.29	0.70	0.15
Glucose, mg/dL	238.2	233.2	255.7	7.7	0.56	0.13	0.17
Triglyceride, mg/dL	924.2	895.8	920.2	12.0	0.30	0.82	0.09
Cholesterol, mg/dL	131.0	158.5	130.7	21.6	0.61	0.99	0.31
HDL-C, mg/dL	44.0	48.0	45.5	6.4	0.72	0.87	0.68
VLDL, mg/dL	184.8	179.9	184.3	2.4	0.32	0.88	0.11
Total protein, mg/dL	4.92	4.55	5.23	0.23	0.94	0.35	0.09
Albumin, mg/dL	1.77	1.55	1.88	0.09	0.71	0.38	0.03
Globulin, mg/dL	3.15	3.00	3.35	0.19	0.91	0.42	0.25

<sup>&</sup>lt;sup>1</sup>Least square means. <sup>2</sup>C vs. E = control vs. enzyme supplementation; L = linear effect of enzyme supplementation; Q: quadratic effect of enzyme supplementation.

albumen width, mm)\*100; yolk index (%) = (yolk height, mm/yolk diameter, mm)\*100; yolk color was determined by using commercially available yolk color fan according to the CIE standard colorimetric system (Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland) and Haugh unit = 100\*log(AH + 7.57-1.7\*EW<sup>0.37</sup>), where AH = albumen height, mm and EW = egg weight, g.

Statistics: The experimental diets were allocated in a complete randomized block design. The cage location (3-tier facing either window or corridor) was considered as a blocking factor. One-way ANOVA was employed using the Mixed Procedure as repeated measures with time being subplot (SAS, 1998). The linear model to test the effects of the experimental diets on laying performance, metabolic profile and egg quality parameters was as follows:  $Y_{ijk} = \mu + B_i + TRT_j + T_k +$  $(TRT*T)_{jk} + e_{ijk}$ , where  $Y_{ijk} = response variable$ ,  $\mu =$ population mean, B<sub>i</sub> = block (I = 1 cage at lower level by corridor side to 6 cage at upper level by window side), TRT; = experimental diet (j = 0 to 2<sup>nd</sup> level of the multienzyme supplement, Tk = time (k = d, wk, or mo relative to initiation of the experiment), (TRT\*T)<sub>ik</sub> = i<sup>th</sup> level of the multi-enzyme supplement by jth time interaction and eik = experimental error. Because of lacking significant effect on response variables, diet by time interaction effect was omitted from the linear model. The time effect was also omitted from the linear model for statistical

analyses of metabolic profiles because blood samples were obtained only at the end of the experimental period. Orthogonal (0 vs. average of 1 and 2 g/kg the multienzyme supplement) and polynomial contrast (linear and quadratic effect of increasing level of the multienzyme supplement) options were computed to evaluate the nature of responses to increasing level of the multienzyme supplement. Statistical significance was declared at P < 0.05.

#### Results

Laying performance: Table 2 summarizes the effect of multi-enzyme supplementation on laying performance parameters. Body weights prior to and after the experiment did not differ across the experimental diets. Regardless of the dietary treatments, all hens gained 4.3% weight relative to initial BW. Neither hen-day egg production nor egg weight responded to the experimental diets. Both parameters, however, changed during the experimental period (time effect, P < 0.0001 for both); the mean values were 84.5, 90.6, 91.5 and 92.4% for hen-day egg production and 60.3, 59.2, 61.3 and 62.3 g for egg weight on month 1, 2, 3 and 4 relative to initiation of the experiment. Hens fed the control diet consumed more feed than hens fed the multi-enzyme supplemented diets (131.5 vs. 124.2 g, P < 0.001). In response to increasing level of multi-enzyme supplementation, feed intake quadratically decreased (P < 0.007). Moreover, feed intake fluctuated over time; the

mean feed intake was 118.9, 115.1, 126.5 and 122.8 g on month 1, 2, 3 and 4 relative to initiation of the experiment. Similarly, feed conversion for hens fed the control diet was worse than for hens fed the multi-enzyme supplemented diets (2.50 vs. 2.35, P < 0.007). Feed conversion ratio linearly decreased with increasing level of multi-enzyme supplementation (P < 0.03). As the experiment advanced, FCR changed (P < 0.001); the mean FCR values were 2.34, 2.18, 2.27 and 2.14 on month 1, 2, 3 and 4 relative to initiation of the experiment.

Metabolic profile: The changes in metabolic parameters in response to the experimental diets are presented in Table 3. There was neither orthogonal nor polynomial effect of multi-enzyme supplementation on amylase activity and blood metabolites, except for serum albumin. As level of multi-enzyme supplementation increased, serum albumin concentration quadratically decreased.

**Egg quality:** As can be seen from Table 4, except for yolk index, none of the egg quality parameters was affected by the dietary treatments. Yolk index for hens fed the control diet was lower than hens fed the multi-enzyme supplemented diets (42.1 vs. 43.2%, P < 0.03) and it decreased linearly with increasing level of supplemental multi-enzyme (P < 0.02). Except for eggshell thickness, other egg quality parameters changed as the experiment continued. On month 1, 2, 3 and 4 relative to initiation of the experiment, the mean values were 75.6, 75.5, 74.8 and 74.1% for shape index (P < 0.01), 3.03, 2.34, 2.67 and 2.72 kg/cm<sup>-2</sup> for eggshell strength (P < 0.02), 10.1, 11.3, 10.7 and 10.1 for yolk color (P < 0.0001), 43.5, 41.0, 43.5 and 43.4% for yolk index (P < 0.0001), 9.7, 8.5, 8.9 and 9.0% for albumen index (P < 0.01) and 85.8, 81.8, 83.8 and 83.2 for Haugh unit (P < 0.05), respectively.

#### Discussion

Enzymes are substrate-specific and play a role in cleaving linkages in NPS to increase nutrient availability from cereal grains for digestion and subsequent absorption. The adverse effects of NPS on utilization of nutrients and nutrient partitioning seem to depend on the amount consumed (Broz, 1993). Each kilogram of barley, rye and wheat contain 30-60 g (1-3),(1-4)- $\beta$ glucan, 100 g arabinoxylan (1-4)-β-xylan with repeated unit of arabinose) and 50-80 g pentosans (1-4)-β-xylan with repeated unit of xylose), respectively (Choct and Annison, 1992b). Al Bustany and Elwinger (1988) added β-glucanase into diets containing barley, oats, wheat, or unprocessed rapeseed and reported no benefit from of enzyme, regardless of Supplementation of a multi-enzyme (1 g/kg) to diet in which barley was replaced with oats up to 100% improved apparent nutrient digestibility and feed conversion in laying hens, despite a dramatic increase in dietary fiber content (Aimonen and Näsi, 1991).

However, positive effects of a mixture of ß-glucanase and pentosonase (0.2 and 0.4 g/kg) supplementation in the diets of chicks containing different cereal grains were not observed (Brenes et al., 1993b). Jaroni et al. (1999) also reported no effect of multi-enzyme supplementation in late producing hens. A multi-enzyme complex mixture (0.5 g/kg) including amylases, cellulases, β-gluconases, lipases and proteases failed to alleviate depression in egg production of hens fed barley-based diets (Benabdeljelil and Arbaoui, 1994). Effects of enzyme supplementation on laying performance of hens fed legume seed-based diets are controversial, but not soybean-based diets. It was shown that a multi-enzyme mixture (fungal β-glucanase, hemicellulase, pectinase, endogluconase, protease and galactosidase) alleviated the adverse effects of alkaloids and toxic compounds of lupines on growth performance (Brenes et al., 1993a). Multi-enzyme supplementation improved performance (FCR) when chickens fed diet containing up to 15% faba beans (Viveros et al., 1993), but not when those fed lupines (Annison et al., 1996). Marsman et al. (1997) suggested that improvement in the nutritional value of soybeans could be achieved with protease and carbohydrase enzyme supplementation. To improve the utilization of soybean meal, Wu et al. (2005) supplemented diets with β-mannanase and reported that despite an increase in egg production, feed intake and egg weight did not change. In the present study, NPS content of feedstuffs was not measured and enzyme supplementation positively affected only feed intake and feed conversion (Table 2), which could be attributed to enhanced nutrient utilization. Multi-enzyme supplementation was shown to increase ileal digestibility of soybean meal (Douglas et al., 2000; Zanella et al. (1999) and corn (Wyatt et al., 1999; Zanella et al., 1999). In another experiment, a mixture of fungal β-glucanase and xylanase supplementation up to 0.25 mg per kilogram was shown to increase egg production and nutrient availability and decrease intestinal viscosity in laving hens, without altering feed intake (Lazaro et al., 2003). Moreover, Mathlouthi et al. (2003) compared the effect of xylanase (560 IU) and β-glucanase (2800 IU) in laying hens fed either wheat-barley or corn-soybean meal based diets. Enzyme supplementation did not affect egg production and egg weight, but improved feed conversion (kilogram feed intake per dozen egg production) in hens fed corn-soybean meal based diet and decreased intestinal viscosity. In a similar experiment, Jackson et al. (1999) evaluated the effect of  $\beta$ -mannanase supplementation (110 IU) on laying performance of hens fed corn-soybean meal based diets providing two different energy levels and reported that β-mannanase supplementation increased egg production and egg weight regardless of the energy level during peak and late-production period.

In a 2x2 factorial arrangement of protein (192 vs. 227

Table 4: The effects of enzyme supplementation on egg quality of hens during the peak period

	Level of enzyme <sup>1</sup> , g/kg				Contrasts², P <		
Parameter	0	1	2	 SEM	 C ∨s. E	L	Q
Shape index, %3	74.8	74.8	75.4	0.3	0.50	0.23	0.47
Shell strength, kg cm <sup>-2 4</sup>	2.70	2.55	2.80	0.13	0.93	0.52	0.20
Shell thickness, mm 10 <sup>-2</sup>	0.391	0.387	0.388	0.004	0.49	0.60	0.63
Yolk colour <sup>5</sup>	10.7	10.6	10.5	0.2	0.45	0.35	0.91
Yolk index, %5	42.1	43.1	43.3	0.4	0.03	0.02	0.38
Albumen index, %3	8.9	9.2	9.0	0.2	0.52	0.79	0.41
Haugh unit <sup>6</sup>	83.3	84.4	83.3	2.4	0.63	0.99	0.34

Least square means. 2C vs. E = control vs. multi-enzyme supplementation; L = linear effect of multi-enzyme supplementation;

g/kg) and enzyme (0 vs. 0.5 g/kg) levels, Pettersson et al. (1990) reported that a mixture β-gluconase and arabinoxylanase improved growth performance of broilers fed a low-protein diet more than those fed a high-protein diet, suggesting improved bioavailability of nutrients. Çiftci et al. (2003) and Scheideler and Abudabos (1998) however reported no energy level by enzyme supplementation interaction effect on laying performance. In a similar experiment, low and highbarlev with and without supplementation was shown to result in decreased dry matter content of digesta and increased starch and nitrogen availability in the small intestine (Hesselman and Åman, 1986). As opposed to expectation that enzymes would increase availability of nutrients, metabolite profile did not change in this experiment (Table 3). In chicks, it was shown that infusion of amino acids, fatty acids and glucose increased the surge of pancreatic digestive enzyme secretion (Yang et al., 1989). Moreover, Ritz et al. (1995a, 1995b) conducted two consecutive experiments and reported that a mixture of amylase and xylanase supplementations increased endogenous enzyme secretion and capacity of the gastrointestinal tract for digestion and absorption.

With the exception of the biochemical composition of diets, the age and breeds of hens do not have a significant effect on physical egg quality parameters that were measured (Aimonen and Uusi-Rauva, 1991; Izat et al., 1985, 1986). In agreement with this conclusion, egg quality changed as age advanced in the present experiment. Earlier works by Berg (1959, 1961) showed no changes in Haugh unit and yolk color in response to diets containing barley with supplemental enzyme. Brenes et al. (1993b) reported that multi-enzyme (0.2 g  $\beta$ -glucanase and 0.4 g pentosonase per kg) supplementation also did not affect egg weight and Haugh unit. However, Aimonen and Uusi-Rauva (1991) supplemented laying hens with multi-enzymes (1 g/kg) for 6 months and reported an increase in yolk color, a decrease in shell thickness and no changes in Haugh unit and albumen weight. Benabdeljelil and Arbaoui (1994) fed hens up to 35% barley-based diets and reported no changes in albumen and shell quality, but

decreased yolk color. No alterations in inner and outer egg quality parameters in response to  $\beta$ -glucanase supplementation were also reported (Al Bustany and Elwinger, 1988).

In conclusion, this experiment was conducted to determine whether multi-enzyme supplementation to laying hens fed corn-soybean meal based diets during the peak period (from week 30 to 46) is beneficial. Except for feed intake and feed conversion, the multi-enzyme supplementation did not affect other laying performance parameters. Except for serum albumin and yolk index, the effect of multi-enzyme on metabolic profile and egg quality parameters was lacking. Decreased feed intake and improved feed conversion could be attributed to enhanced utilization of nutrients. In consideration with cost analysis, supplementation of multi-enzyme may allow profitable egg production.

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Q: quadratic effect of multi-enzyme supplementation. Time effect (P < 0.01). Time effect (P < 0.02). Time effect (P < 0.001).

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