Reseach Note

The Effects of Supplementation of Humate and Probiotic on Egg Production and Quality Parameters During the Late Laying Period in Hens

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ABSTRACT This study was designed to investigate whether inclusions of humate and probiotic into diets of hens during the late laying period increases egg production and improves egg quality. Hisex Brown layers (n = 300), 54 wk of age, were fed a control diet, 0.1% humate, 0.2% humate, 0.1% probiotic, or 0.2% probiotic for 75 d. Active ingredients of humate and probiotic were polymeric polyhydroxy acids (humic, fulvic, ulmic, and humatomelanic acids) and bacterial cultures (*Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Enterococcus* spp.), respectively. Egg production and feed intake were measured daily, and egg weight was measured biweekly. Also, a sample of 12 eggs from each group was collected randomly to determine egg quality every 25 d. The data were analyzed as repeated measures with time as subplot. There were no effects of dietary treatments on feed intake and egg weight. Egg production for hens supplemented with humate and probiotic was not different but was greater than for control hens. Egg production increased linearly and mortality and feed conversion efficiency (weight of feed / weight of eggs) decreased linearly with increasing levels of supplemental humate and probiotic. There were no effects of treatments on egg quality. In conclusion, supplementation of humate and probiotic during the late laying period increased egg production, reduced mortality, and improved feed conversion efficiency but did not improve egg quality.

(*Key words*: probiotic, humate, egg production, egg quality, late laying period)

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INTRODUCTION

To enhance nutrient utilization, improve feed conversion efficiency, and maintain health status, inclusion of probiotics and humates in rations is preferable to antibiotics, primarily because they cause no harmful effects on consumers (Onifade et al., 1999). Humates, a part of fertilizers, are derived from plant matter decomposed by bacteria (Senn and Kingman, 1973) and contain humus, humic acid, fulvic acid, ulmic acid, and some microelements (Stevenson, 1994). Previous studies with respect to humates have focused mainly on the growth of germinal tissue in seed. The idea of using humates as feed additives in animal nutrition is new. At first, humates were used as a part of replacement therapy for digestive system disturbances such as malnutrition and diarrhea and increased for feed conversion efficiency in calves, dogs, and cats (Kühnert et al., 1989, 1991). Remarkable changes in electrolyte balance and enhancements in immune potency of ruminants (Lenk and Benda, 1989; Griban et al., 1991) and poultry (Parks et al., 1986) in response to humate supplementation have been reported. Moreover, consistent agreements in the limited number of published articles show that humates promote growth by altering partitioning of nutrient metabolism (Stepchenko et al., 1991; Zhorina and Stepchenko, 1991; Parks, 1998), and reducing mortality (Eren et al., 2000), and improving feed conversion efficiency (Shermer et al., 1998; Eren et al., 2000).

Probiotic is a generic term, and products can contain yeast cells, bacterial cultures, or both that stimulate microorganisms capable of modifying the gastrointestinal environment to favor health status and improve feed efficiency (Dierck, 1989). Mechanisms by which probiotics improve feed conversion efficiency include alteration in intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients (Yeo and Kim, 1997). Therefore, the major outcomes from using probiotics include improvement in growth (Yeo and Kim, 1997), reduction in mortality (Kumprecht and Zobac, 1998), and improvement in feed conversion efficiency (Yeo and Kim, 1997).

Numerous studies have been conducted to determine the effect of biotechnological products on performance during the growing period in broilers. To our knowledge, the effect of growth stimulators during the late laying period has not been tested. Therefore, the objectives of

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this study were to investigate the effects of supplementation of humate and probiotic on egg production and egg quality parameters of hens during the late laying period and compare the effectiveness of supplemental humate and probiotic.

MATERIALS AND METHODS

Birds, Diet, and Management

The Research Animal Ethic Committee of Atatürk University approved this experimental protocol. Three hundred Hisex Brown layers, 54 wk of age and with uniform BW, were placed into cages (50 × 46 × 46 cm). They were then assigned randomly to be fed 1 of 5 isocaloric and isonitrogenous experimental diets: a control diet containing neither humate nor probiotic and diets containing either humate[2] (0.1 and 0.2%) or probiotic[3] (0.1 and 0.2%). Each treatment was replicated in 12 cages with 5 hens each. Each kilogram of humate contained 160 mg polymeric polyhydroxy acids (humic, fulvic, ulmic, and humatomelanic acids), 663.3 mg SiO_2, and other minerals (Mn, 50 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.5 mg; and Al, Na, K, Mg, and P in trace amounts). Each kilogram of probiotic contained *Lactobacillus plantarum* (1.89×10^{10} cfu), *Lactobacillus delbrueckii* subsp. *bulgaricus* (3.09×10^{10} cfu), *Lactobacillus acidophilus* (3.09×10^{10} cfu), *Lactobacillus rhamnosus* (3.09×10^{10} cfu), *Bifidobacterium bifidum* (3.00×10^{10} cfu), *Streptococcus salivarius* subsp. *thermophilus* (6.15×10^{10} cfu), *Enterococcus faecium* (8.85×10^{10} cfu), *Aspergillus oryza* (7.98×10^{9} cfu), and *Candida pintolopesii* (7.98×10^{9} cfu). The experimental diets (Table 1) were formulated to meet or exceed the NRC recommendations (NRC, 1994). During the experiment (75 d), hens were fed ad libitum once daily at 0730 h; water was available all the times, and a photoperiod of 17 h was maintained.

Sample Collection and Analytical Procedure

Feed samples were collected monthly and analyzed for DM and CP contents (AOAC, 1990). Energy, Ca, and P contents of the experimental diets were calculated from tabular values of feedstuffs for chickens (Jurgens, 1996). Feed intake and egg production were measured daily. Samples of eggs collected biweekly were stored for 24 h at room temperature and then weighed. Feed conversion efficiency was expressed as kilograms of feed consumed per kilogram of egg produced. An additional sample of 12 eggs was randomly collected from each experimental group every 25 d to assess egg quality parameters (Yörük and Bolat, 2003). Egg quality parameters were specific gravity, shape index, shell stiffness, shell thickness, yolk color[4], albumen index, yolk index, and Haugh units.

Statistics

The BW data obtained prior to initiation of the experiment were used as a covariate for statistical analyses of all response variables. The ANOVA was conducted using the mixed procedure (SAS Institute, 1998) as repeated measures with time as the subplot. The linear model to test the effects of the experimental diets on egg production and quality parameters was as follows:

$$Y_{aijk} = \mu + b_0 + b_1(Cov_a) + B_i + TRT_j + \text{error A} + t_k + (TRT \times t)_{jk} + \text{error B}$$

where Y_{aijk} = response variable, μ = population mean, b_0 = intercept, b_1 = slope, Cov_a = covariate (a = BW), B_i = block (i = cage 1 at lower level by corridor side to cage 6 at upper level by window side), TRT_j = experimental diet (j = 0 to 2 levels of supplemental H and P), error A = whole plot error, t_k = time (k = d or wk relative to initiation of the experiment), $(TRT \times t)_{jk}$ = experimental diet j and time k interaction, and error B = subplot error. Moreover, we constructed orthogonal contrasts to compare the mean response variables for hens fed the control diet vs. hens fed humate or vs. hens fed probiotic as well as hens fed humate vs. hens fed probiotic and polynomial contrast to determine the nature of response variables to increasing levels of supplemental humate and probiotic. The effects of the experimental diets on response variables were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

Production Performance

Mortality for hens fed the control diet was not different from that for hens fed humate and probiotic diets. The mortality was also not different among hens fed humate and probiotic diets. There was also no effect of increasing the level of supplemental humate on mortality, whereas there were linear and quadratic decreases in mortality with increased supplemental probiotic (Table 2). Deaths occurred toward the end of the experiment, and mortality was 0.18, 0.26, 1.78, 0.88, and 1.08% on d 15, 30, 45, 60, and 75, respectively, relative to initiation of the experiment (time effect, $P < 0.0001$). Autopsy findings revealed that deaths were related to noninfectious causes. Little is known about the mechanism by which humate supplementation enhances the life span and improves production efficiency. However, available data consistently suggest that humate supplementation may benefit poultry production. Pukhova et al. (1987) reported that supplementation of Na humate in rats exposed to lethal doses of radioactivity increased the life span. In similar studies, it was shown that after high doses, supplemental humate alleviates toxicity of Cr in fish (Stackhouse and Benson,

[2]Farmagülatör DRY Humate, Farmavet International Inc., Kocaeli 41400, Turkey.
[3]Protexin Compounder, Novaritis Inc., Instanbul 80700, Turkey.
[4]Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland.

TABLE 1. Ingredient and chemical composition of the experimental diets

	Control	Humate (%)		Probiotic (%)	
Ingredient, %	0	0.1	0.2	0.1	0.2
Corn	45.00	45.00	45.00	45.00	45.00
Soybean meal, 44% CP	21.00	21.00	21.00	21.00	21.00
Wheat	7.00	7.00	7.00	7.00	7.00
Barley	3.05	3.05	3.05	3.05	3.05
Wheat bran	9.50	9.40	9.30	9.40	9.30
Molasses	2.00	2.00	2.00	2.00	2.00
Sunflower oil	1.00	1.00	1.00	1.00	1.00
Limestone	9.50	9.50	9.50	9.50	9.50
Dicalcium phosphate[1]	1.00	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30	0.30
Vitamin-mineral premix[2]	0.35	0.35	0.35	0.35	0.35
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Antioxidant[3]	0.10	0.10	0.10	0.10	0.10
Humate[4]	—	0.10	0.20	—	—
Probiotic[5]	—	—	—	0.10	0.20
Calculated content					
DM, %	89.21	88.99	89.09	89.27	88.96
ME, kcal/kg DM[6]	2,530.00	2,530.00	2,530.00	2,530.00	2,530.00
CP, %	15.66	15.61	15.53	15.57	15.58
Ca,[6] %	3.86	3.86	3.86	3.86	3.86
P,[6] %	0.61	0.61	0.61	0.61	0.61

[1]Each kilogram contained: Ca, 24%; P, 17.5%.

[2]Each kilogram contained: vitamin A, 15,000 IU; cholecalciferol, 1,500 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_{12}, 15 μg; 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.

[3]Ethoxyquin.

[4]Each kilogram contained: 160 mg polymeric polyhydroxy acids (humic, fulvic, ulmic, and humatomelanic acids), 160 mg; SiO_2, 663.3 mg; and other minerals (Mn, 50 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.5 mg, and Al, Na, K, Mg, and P; traced amounts). Farmagülatör DRY™ Humate, Farmavet International Inc., Kocaeli, Turkey.

[5]Each kilogram contained: *Lactobacillus plantarum*, 1.89×10^{10} cfu; *Lactobacillus delbrueckii* subsp. *bulgaricus*, 3.09×10^{10} cfu; *Lactobacillus acidophilus*, 3.09×10^{10} cfu; *Lactobacillus rhamnosus*, 3.09×10^{10} cfu; *Bifidobacterium bifidum*, 3.00×10^{10} cfu; *Streptococcus salivarius* subsp. *thermophilus*, 6.15×10^{10} cfu; *Enterococcus faecium*, 8.85×10^{10} cfu; *Aspergillus oryza*, 7.98×10^{9} cfu; *Candida pintolopesii*, 7.98×10^{9} cfu. Protexin Compounder, Novaritis Inc., Istanbul, Turkey.

[6]Calculated from tabular values of feedstuffs for chickens (Jurgens, 1996).

1989) and Cd in chickens (Herzig et al., 1994) by reducing deposition of toxic metals in organs. Supplementation with probiotics has also been shown to enhance survival by altering gastrointestinal flora (Netherwood et al., 1999) to suppress growth of pathogenic bacteria (Ehrmann et al., 2002) and by enhancing immune potency (Balevi et al., 2001).

No effects of the experimental diets on feed intake were noted (Table 2). However, feed intake was affected by time ($P < 0.0001$), with averages of 117.7, 116.3, 124.1, 138.6, and 126.3 g on d 15, 30, 45, 60, and 75, respectively, relative to initiation of the experiment. The mechanism by which humate affects poultry performance is largely unknown, whereas it is well established that probiotics alter gastrointestinal pH and flora to favor an increased activity of intestinal enzymes and digestibility of nutrients (Dierck, 1989). Schneitz et al. (1998) reported that there were no changes in intestinal pH and volatile fatty acid concentrations, but there were linear increases in digestibility of nutrients in hens supplemented with increasing levels of probiotic. In similar studies involving broilers, despite a lack of feed intake data, it was reported that supplementation of humate (Kocabağlı et al., 2002) and probiotic (Jin et al., 1998) did not alter feed conversion efficiency on d 21, but improved it on d 42. It appears that supplementations of humate and probiotic do not improve growth by affecting feed intake per se, suggesting that improvement in weight gain and reduction in feed conversion efficiency by supplemental humate and probiotic could be related to their promoting effects on metabolic processes of digestion and utilization of nutrients (Yeo and Kim, 1997). Also, in this study, a lack of effects of the experimental diets on feed intake could be related to the completion of the growing process at this age. In relation to this, there could be less variation in gastrointestinal tract capacity of older hens.

Egg production for hens fed humate and probiotic diets were not different, but egg production for both groups was greater than egg production for hens fed the control diet (70.0 vs. 63.7% and 69.0 vs. 63.7%, respectively, Table 2). Moreover, there were linear increases in egg production with increased supplemental humate and probiotic. Egg production was also affected by time ($P < 0.0001$) with averages of 61.6, 65.6, 70.0, 72.6, and 71.8% on d 15, 30, 45, 60, and 75, respectively, relative to initiation of the experiment. To our knowledge, no egg production data

TABLE 2. The effects of supplementation of humate and probiotic on performance and production parameters of hens during the late laying period

	Least square mean						Statistical contrast,[2] $P > F$						
	Control	Humate (%)		Probiotic (%)			Orthogonal			Polynomial			
Parameter	0	0.1	0.2	0.1	0.2	SEM	C vs. H	C vs. P	H vs. P	LH	QH	LP	QP
Mortality,[3] %	1.13	0.60	0.83	1.33	0.27	0.3	0.26	0.46	0.63	0.28	0.13	0.002	0.01
Feed intake,[3] g	123.9	127.8	125.0	122.6	123.7	2.3	0.27	0.99	0.17	0.70	0.20	0.97	0.72
Egg production,[3] %	63.7	70.0	70.0	68.2	69.7	1.7	0.002	0.01	0.53	0.003	0.08	0.04	0.55
Egg weight, g	66.7	68.2	67.3	67.4	68.0	0.6	0.17	0.18	0.97	0.41	0.06	0.16	0.97
FCE[4, 5]	2.97	2.70	2.68	2.82	2.63	0.09	0.01	0.02	0.69	0.005	0.15	0.02	0.88

[1]Control = hens not supplemented with probiotic or humate.

[2]Statistical contrast: C vs. H = contrasting hens not supplemented with humate versus hens supplemented with humate; C vs. P = contrasting hens not supplemented with probiotic vs. hens supplemented with probiotic; H vs. P = contrasting hens supplemented with humate versus hens supplemented with probiotic; LH = linear effect of humate supplementation; QH = quadratic effect of humate supplementation; LP = linear effect of probiotic supplementation; QP = quadratic effect of probiotic supplementation.

[3]Time effect ($P < 0.0001$).

[4]FCE = feed conversion efficiency (kg feed consumed / kg egg produced).

[5]Time effect ($P < 0.04$).

in response to supplemental humate are available. Alteration in nutrient partitioning (Parks, 1998; Stepchenko et al., 1991; Zhorina and Stepchenko, 1991) could be associated with increased egg production in hens receiving supplemental humate. Mohan et al. (1995) reported a quadratic increase in egg production in chickens supplemented with 0, 100, and 150 mg probiotic (*Lactobacillus*, *Bifidobacterium*, *Aspergillus*, and *Torulopsis* spp. at 27×10^9 cfu / 10 g) per kilogram of diet during the peak period. Based on the results of the present study, differences in the nature of egg production response could be attributed to differences in level of supplemental probiotic and the physiological stage of experiment conducted.

Except for a quadratic increase in response to increased levels of supplemental humate, there were no effects of the experimental diets on egg weight (Table 2). Reductions in feed conversion efficiency and mortality can be considered as criteria of benefits from supplementations of humate and probiotic. There was no difference in feed conversion efficiency of hens fed humate and probiotic diets. However, there were improvements in feed conversion efficiency for hens fed humate (2.69 vs. 2.97) and probiotic (2.73 vs. 2.97) diets compared with hens fed the control diet (Table 2). Moreover, there were linear decreases in feed conversion efficiency when hens were supplemented with increasing levels of humate and probiotic. These results are in agreement with the results of studies involving broilers on supplementations of humate (Stepchenko et al., 1991; Zhorina and Stepchenko, 1991; Kocabağlı et al., 2002) and probiotic (Yeo and Kim, 1997; Jin et al., 1998). Feed conversion efficiency fluctuated over time ($P < 0.04$), with averages of 2.86, 2.62, 2.70, 2.95, and 2.67 on d 15, 30, 45, 60, and 75, respectively, relative to initiation of the experiment.

Egg Quality

Unless mentioned below, there were no effects of the experimental diets on specific gravity (1.075 ± 0.0001 g / L), shape index (77.6 ± 0.4%), shell stiffness (1.23 ± 0.12 kg / cm^2), shell thickness (0.37 ± 0.01 mm), yolk color (9.6 ± 0.2), albumen index (7.7 ± 0.3%), yolk index (43.2 ± 0.5%), and Haugh unit (78.8 ± 1.2) (mean ± SEM). Moreover, specific gravity, shape index, shell stiffness, shell thickness, and yolk color did not change over time. Mohan et al. (1995) reported a slight improvement in eggshell thickness in hens supplemented with probiotic for 10 wk during the peak period. Albumen index ($P < 0.001$), yolk index ($P < 0.01$), and Haugh unit ($P < 0.003$) were affected by time. Average albumen index was 7.4, 8.4, and 7.3%, yolk index was 42.3, 43.8, and 43.6%, and Haugh unit was 78.1, 81.3, and 76.8 on d 25, 50, and 75, respectively, relative to initiation of the experiment. Moreover, there were significant effects of experimental diets by time interaction on albumen index ($P < 0.05$; Figure 1). As the feeding trial progressed, the albumen index for hens fed

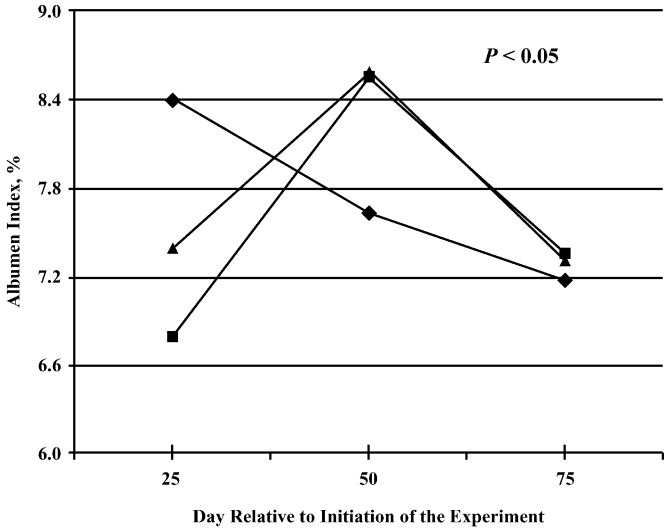

FIGURE 1. The effects of supplementations of humate and probiotic by time interaction on albumen index of hens during the late laying period (—◆—, hens not supplemented with humate and probiotic; —▲—, hens supplemented with humate; and —■—, hens supplemented with probiotic).

the control diet continuously decreased, and those for hens fed humate and probiotic first increased and then decreased. Haugh unit is a function of albumen index (Silversides and Scott, 2001), and both were highly correlated ($r = 0.93$, $P < 0.0001$). Moreover, both parameters are accepted as two major indicators determining egg quality and do not change by dietary regimen but by aging (Silversides and Scott, 2001).

In this study, the effects of supplementation of humate and probiotic during the late laying period on egg production and egg quality parameters were investigated, and their effectiveness was compared. Supplemental humate and probiotic had linear effects on production parameters including reduced mortality and feed conversion efficiency and increased egg production. However, they had no consistent effects on egg quality parameters. In conclusion, supplementation of humate or probiotic may extend the profitability of a layer flock.

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