







RESEARCH ARTICLE

Enhanced Egg Weight, Egg Production and Shell Breaking Strength in Late Laying Period of Hens Fed a Diet Containing a Eubiotic Mixture

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ABSTRACT

The effects of different levels of an eubiotic on laying performance, egg quality parameters, serum enzymes and antioxidant levels, and egg yolk fatty acid composition were examined in the present study. Six diets were formulated to contain 0, 200, 400, 600, 800 and 1000 mg/kg EFA. Each diet was randomly fed to a group of 24 hens for 10 weeks, housed in 6 separate cages (4 hens per cage). Average egg weight was remarkably increased as an effect of EFA dietary supplementation. In comparison to the control group (89.2%), significantly higher egg production rates of 93.7 and 96.7% were observed in the groups of hens fed diets supplemented with 800 and 1000 mg/kg EFA, respectively. An improvement of 5 to 11% in FCR of EFA supplemented groups was found. Concerning the other examined parameters, only shell breaking strength was increased by 30-36% in EFA supplemented groups at the level of 200, 800 and 1000 mg/kg, whereas no significant differences in egg yolk fatty acid composition, serum enzymes and antioxidant levels were observed among groups. In conclusion, an improved hen performance at late laying phase could be achieved as an effect of EFA dietary supplementation at the level of 1000 mg/kg.

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Introduction

The use of antibiotic growth promoters (AGPs) in animal nutrition was banned in 2006 by the EU. Since then a wide range of feed additives such as organic acids, probiotics, prebiotics, essential oil compounds (EOC), minerals and trace elements have been used as alternatives to AGPs with positive claims of an improved gut microflora and animal performance (Huyghebaert et al., 2011; Gadde et al., 2017).

Active agents from certain feed additives have been recently isolated and combined in single products to offer

improved animal health and performance. A dietary supplementation of a mixture containing EOC (thymol, eugenol and piperine) and an organic acid at the level of 300 mg/kg enhanced performance parameters in broiler chickens (Weber et al., 2012) and turkey poults (Giannenas et al., 2014). The purpose of mixing such active agents is to create a eubiosis synergistic effect to optimally balance gut microbiota in the absence of AGPs. It was recently shown that a mixture of EOC and organic acid increased live weight gains and reduced *E. coli* fecal shedding in piglets (Torallardona et al., 2016). A possible improvement in hen performance during late laying

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period as an effect of such dietary interventions is of great importance in sustainable animal production systems. The effects of single or multiple mixture of functional feed materials or active substances (symbiotic/eubiotic effect) have already been examined in laying hens: Supplementing the diets with dry whey powder at level of 60g/kg for 13 weeks significantly reduced ceecal counts of *Clostridium perfringens* and increased egg production from 75% to 82.5% in late laying phase (Pineda-Quiroga et al., 2017). However, dietary supplementation with xylooligosaccharides known as xylose polymer or prebiotic did not affect laying performance, but improved eggshell quality and digestibility of dietary calcium as well as reduced plasma cholesterol level (Li et al., 2017). A diet containing 4% of a mixture of red seaweeds, *Chondrus crispus* known as Carrageen and a prebiotic had a protective effect against *Salmonella* Enteritis colonisation in laying hens (Kulshreshtha et al., 2017). The humate (humic, fulvic, ulmic, and humatomelanolic acids) and probiotic together in the diets of laying hens during late laying period increased egg production and improved feed conversion efficiency (FCR) without any effect on egg quality (Yoruk et al., 2004). A mixture of additives containing both *Aspergillus awamori* and lactic acid bacteria had no effects on the performance of laying hens, but significantly increased unsaturated and reduced saturated fatty acid contents in the egg yolk (Saleh et al., 2017). Finally, dietary supplementation with selenium enriched yeast products could be more efficient in increasing selenium bioavailability of whole egg compared to the inorganic selenium sources (Chantiratikul et al., 2017).

Feed materials/additives that are produced through fermentation processes contain several active substances and have been reported to improve performance and health in broiler and Japanese quails (Yasar and Gok, 2014; Yasar et al., 2016). An EFA produced at our laboratory through a solid-state fermentation (SSF) process containing enzymes, organic acids and live yeast cells successfully induced a growth promoting effect (GPE) in broiler chicken (Yasar and Yegen, 2017), and a similar SSF product containing fermented whey, fermented grain and fermented fruit pomace had a significant positive effect on health status and performance in both calves and lambs with severe diarrhea at a daily dose of 15 g for a period of 2 to 5 days (Yasar et al., 2017). Many studies with dietary supplements have already been implemented with the intention to improve laying performance of hens in late laying period (Yoruk et al., 2004 and Bolukbasi et al., 2010; Kaya et al., 2014a; Świątkiewicz et al., 2018). To the best of our knowledge, no studies exist concerning the effects of a SSF additive containing several active agents on the productive parameters of in laying hens.

Therefore, this study aimed at highlighting the effects of various dietary levels of a eubiotic feed additive (EFA) containing organic acids, probiotic yeast/bacteria, enzymes and rosemary dry powder on performance, egg quality, egg yolk fatty acid composition, serum enzymes and antioxidant levels in 75 weeks old Lohman LSL hens.

Materials and Methods

Animal and Housing

The research was conducted at the poultry research station of Atatürk University, Erzurum, Turkey in accordance with EU Directive 2010/63/EU. A total of 144, 75 week-old Lohman hens were randomly allocated into 6 treatment groups. Each group included 6 cage replicates of 4 hens (50 x 46 x 46). The hens were previously fed with the control diet (Table 1) and subjected to a lighting regime of 16 h per day prior to the trial.

Table 1. Composition of basal diet

Ingredients (ground)	Composition (g per kg)
Corn	520.00
Soybean meal	240.00
Wheat	118.63
Limestone	90.80
Vegetable oil	10.30
Dicalcium phosphate	11.54
Vit+Min mixture*	2.00
NaCl	5.00
DL-Methionine (99% purity)	1.23
L-lysine	0.50
Total	1000.00
Chemical Analysis (g per kg)	
Crude protein	167.00
Calcium	37.80
Total phosphorous	6.75
Calculated Analysis (g per kg)	
Metabolisable Energy (Kcal per kg)	2752
Lysine	9.00
Met+Cys	6.20

* Supplied per kilogram of diet: 12 000 IU vitamin A; 2 500 IU vitamin D3; 30 IU vitamin E; 4 mg vitamin K3; 3 mg vitamin B1; 6 mg vitamin B2; 30 mg niacin; 10 mg calcium D-pantothenate; 5 mg vitamin B6; 0.015 mg vitamin B12; 1 mg folic acid; 0.050 mg D-biotin; 50 mg vitamin C; 300 mg choline chloride; 80 mg manganese; 60 mg iron; 60 mg zinc; 5 mg copper; 0.5 mg cobalt; 2 mg iodine; 0.15 mg selenium.

Dietary Treatments, Sample Collection and Analysis

The additive used in the study was developed by a SSF process of agricultural co-products at the department of Animal Science, Agricultural Faculty of Iğdir University, Turkey. Each g of the product contained 12.5 mg of finely powdered leaves of rosemary (*Rosmarinus officinalis*), 3.1×10^9 c.f.u (colony forming unit) of *Saccharomyces cerevisiae*, a 3.7×10^9 c.f.u of *Streptococcus thermophilus*, a 1.1×10^8 c.f.u of *Lactobacillus spp.*, an activity of 40 IU of amylase, 845 IU of betaglucanase, 2000 of IU arabinoxylanase and 15% of organic acids (mostly acetate, lactate and citrate). The EFA has a pH of 3.6-3.9, an average particle size of 1-2 mm, and a light orange colour. Six experimental diets (mash) were prepared in the present study. Control diet (Table 1) was formulated to meet the nutrient requirements of laying hens (NRC, 1994). Experimental diets were further supplemented with 200, 400, 600, 800 and 1000 mg of EFA. The trial lasted 70 days, and during this period all the hens had free access to water and feed and were subjected to a photoperiod of 16 h per day.

Feed intake, egg weight, egg production, FCR (g feed per g egg produced per group) were daily recorded for each cage, while the egg weight, Haugh unit, shell breaking strength, shell thickness, shape index and ratio of albumen, yolk and shell were determined biweekly using six eggs from each dietary treatment. At the end of the study, blood samples (n = 6/group) were collected from wing vena into additive free blood tubes. The levels of glutathione peroxidase (GPx), malondialdehyde (MDA), total antioxidant (TAS) and total oxidants (TOS) in the blood serum of the hens were analysed by the use of commercial kits (Roche) in auto-analyser (Cobas 6000, Japan). In addition, the fatty acid composition was examined in the samples of egg yolk (Folc, 1957; AOAC 2000). Fatty acid methyl esters were separated using gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA) equipped with a fused silica capillary column (25 m by 0.2 mm) with 5% cross-linked phenylmethyl silicone. Fatty acid methyl ester profiles of the samples were identified by comparing the commercial Eucary database with the MIS software package (MIS ver. No 3.8, Microbial ID, Inc., Newark, Delaware). Individual fatty acid methyl esters were expressed as percentage of total FA.

Statistical Analysis

Data was analysed by using a general linear model (GLM) to test the effects of EFA supplementation on the laying performance, egg and egg-shell quality parameters, serum enzyme activities and antioxidant capacity, and fatty acid composition of yolk. Differences among treatments means were detected by Duncan's multiple comparisons test at 0.05 significance level using SPSS version 21 for Windows.

Results

As indicated in Table 2, the effect of EFA supplementation on daily feed intake (FI) was not significant ($P > 0.05$). The mean daily FI ranged from 149.02 to 150.41 g in the present study. The effect of EFA supplementation on egg production was significant ($P < 0.05$). Egg production rates were increased in the 800 and 1000 mg/kg EFA supplemented groups (93.75 and 96.66%, respectively) compared to the controls (89.23%). Egg production rates of the other groups did not vary. The egg weight of the control group (64.13 g) was significantly lower than that of the 200 and 1000 mg/kg EFA supplemented groups (69.25 and 67.34 g, respectively) ($P < 0.05$). Egg weight of the other EFA groups was not significantly different compared to the controls. FCR was significantly improved ($P < 0.05$) in 200 and 1000 mg/kg EFA supplemented groups (2.39 and 2.32, respectively) (Table 2).

In the present study, the effect of EFA supplementation on shape index, eggshell index, egg yolk index, egg-albumin index, shell thickness and Haugh unit was not significant ($P > 0.05$). However, egg-shell breaking strength was significantly ($P < 0.05$) increased as an effect of EFA dietary supplementation (Table 3; from 1.10 in the controls to 1.46, 1.50 and 1.42 kg/cm³ in the 200, 800 and 1000 mg/kg EFA supplemented groups, respectively). Values of serum GPx, MDA, TAS and TOS levels were not influenced by EFA dietary supplementation (Table 4). As indicated in Table 5, EFA dietary supplementation did not also induce significant effects on fatty acid composition of egg yolk.

Table 2. Effects of varying supplementation levels of EFA on laying performance

EFA, mg/kg	Feed intake (g/hen)	Egg weight (g/hen)	Egg production (% of kept hens)	FCR (g:g)
0	149.02	64.13 ^c	89.23 ^c	2.62 ^a
200	148.77	69.25 ^a	90.41 ^{bc}	2.39 ^{bc}
400	148.11	65.58 ^{bc}	92.70 ^{bc}	2.45 ^{bc}
600	148.15	64.77 ^c	92.08 ^{bc}	2.49 ^{ab}
800	148.54	64.70 ^c	93.75 ^{ab}	2.46 ^{bc}
1000	150.41	67.34 ^{ab}	96.66 ^a	2.32 ^c
SEM ¹	0.24	0.31	0.51	0.02
<i>P</i> -value	>0.05	=0.000	=0.000	=0.000

^{a-c} Values in the same column indicate significant ($P < 0.05$) differences.

¹ SEM; Standard error of means.

Table 3. Effects of varying supplementation levels of EFA on egg quality

EFA (mg/kg)	Shape index (%)	Egg shell index (%)	Egg yolk index (%)	Albumin index (%)	Eggshell breaking strength (kg/mc ³)	Shell-thickness (µm)	Haugh Unit
0	75.75	10.92	29.62	59.45	1.10 ^b	344.50	91.48
200	74.37	10.80	26.33	62.85	1.46 ^a	332.88	93.54
400	76.25	10.89	25.29	63.81	1.30 ^{ab}	332.54	89.95
600	75.31	11.03	26.57	62.39	1.35 ^{ab}	334.00	89.30
800	74.5	11.02	26.76	62.20	1.50 ^a	331.25	86.94
1000	74.41	10.43	26.02	63.54	1.42 ^a	336.66	94.58
SEM ¹	0.33	0.17	0.50	0.48	0.04	1.89	0.91
<i>P</i> -value	>0.05	>0.05	>0.05	>0.05	=0.047	>0.05	>0.05

^{a-c} Values in the same column indicate significant ($P < 0.05$) differences.

¹ SEM; Standard error of means.

Table 4. Effects of varying supplementation levels of EFA on serum glutathione peroxidase (GPx), malondialdehyde (MDA), total antioxidant (TAS, trolox equivalent) and total oxidants (TOS) levels

EFA (mg/kg)	GPx (nmol/ml)	MDA (nmol/ml)	TAS (µmol/ml)	TOS (µmole/L)
0	3.10	15.69	0.61	35.93
200	3.01	19.12	0.63	26.68
400	3.54	20.38	0.67	32.31
600	3.46	19.65	0.67	30.50
800	3.70	17.58	0.55	30.45
1000	3.13	17.05	0.49	30.68
SEM ¹	0.14	0.76	0.03	3.78
<i>P-value</i>	>0.05	>0.05	>0.05	>0.05

^{a-c} Values in the same column indicate significant ($P < 0.05$) differences.

¹ SEM; Standard error of means.

Table 5. Effect of dietary supplementation of EFA on fatty acid composition of yolk

Fatty acids	Control	EFA 200 mg/kg	EFA 400 mg/kg	EFA 600 mg/kg	EFA 800 mg/kg	EFA 1000 mg/kg	SEM ¹	<i>P-value</i>
Miristic	1.24	1.23	1.29	1.21	1.18	1.30	0.04	>0.05
Palmitic	28.73	29.46	32.08	32.90	29.78	29.46	1.01	>0.05
Palmitoleic	5.13	4.92	4.36	4.19	4.92	5.03	0.13	>0.05
Stearic	12.06	13.19	14.33	14.65	15.57	15.24	0.44	>0.05
Oleic	32.77	30.73	27.79	27.33	27.61	29.77	0.73	>0.05
Linoleic	13.28	14.10	14.17	13.75	14.50	12.91	0.55	>0.05
Linolenic	0.64	0.61	0.59	0.60	0.58	0.59	0.02	>0.05
Arachidonic	2.37	2.41	2.31	2.25	2.46	2.40	0.05	>0.05
EPA	1.09	0.85	0.76	0.75	0.85	0.71	0.04	>0.05
DHA	0.96	0.99	0.82	0.79	0.79	0.84	0.03	>0.05
SFA	42.03	43.89	47.70	48.77	46.53	46.01	0.53	>0.05
MUFA	37.90	35.66	32.16	31.53	32.54	34.80	0.81	>0.05
PUFA	18.34	18.97	18.67	18.14	19.20	17.46	0.60	>0.05

¹ SEM; Standard error of means.

Discussion

Feed intake of hens was not affected by EFA dietary supplementation. Several studies demonstrated that the beneficial effects of microbial feed additives (probiotics/eubiotics) in several poultry species were not regulated by a change in voluntary feed intake (Yoruk et al., 2004; Yasar and Yegen, 2017). On the other hand, the use of feed additives in subclinical infected birds remarkably increased FI and performance by improving gut health and the rate of nutrient digestion and assimilation at the digestive sites (Jiang et al., 2010; Kulshreshtha et al., 2017).

It is most likely that the enhanced egg productions and egg weight as well as egg breaking strength due to the EFA dietary supplementation could be a result of a combined eubiosis effect of organic acids, enzymes and probiotics that are present in the examined feed additive. It was previously reported that the dietary supplementation with probiotics till the level of 150 mg/kg diet has a positive quadratic effect on egg production (Mohan et al., 1995). The use of probiotics, synbiotics and eubiotics with or without organic acids under normal or subclinical infection conditions has been shown to provide an optimal balance of gut microbiota, which, in turn, is responsible for an improved performance in laying hens (Yoruk et al., 2004; Gaggia et al., 2010; Kaya et al., 2014b; Torallardona et al., 2016; Gadde et al., 2017; Pineda-Quiroga et al., 2017). The use of exogenous enzymes in laying hen diets is often recommended in order to efficiently utilize the cell wall polysaccharide contents, and positive effects on FCR, egg production and egg yield are generally reported (Mirzaie et al., 2012). It can be concluded that the daily administration of probiotics and organic acids from EFA in our study might

contributed in a healthier gut, and the exogenous enzymes caused a greater breakdown of cell-wall constituents and other nutrients with a further positive effect on laying performance.

Several herbal extracts significantly improved egg production and FCR (Cabuk et al., 2006; Kaya et al., 2013) even in hens reared under extreme cold conditions (Akbari et al., 2016). Egg mass and egg weight also appeared to increase after EO supplementation (Olgun, 2016). The EFA used in the present study contained a powder derived from rosemary leaves. Powder of rosemary leaves used as feed additive at a rate of 0.3 to 0.9% significantly increased both egg weight and egg production (Alagawany and Abd El-Hack, 2015). The products of rosemary powders or extracts are rich in phenolic compounds (carnosol and carnosic acids with antioxidant effects) and are shown to enhance the performance of poultry (Bozin et al., 2006; Yasar et al., 2017). Rosemary essential oils were previously found to reduce yolk percentage and Haugh unit (Bolukbasi et al., 2008), whereas the rosemary powder increases the values of these parameters (Alagawany and Abd El-Hack, 2015). These discrepancies may be related with the dose or the different forms of rosemary (oils or powdered raw leaves). Both forms of rosemary were found to lower serum triglyceride and total cholesterol levels (Bolukbasi et al., 2008; Alagawany and Abd El-Hack, 2015) as well as increase serum superoxide dismutase activity (Alagawany and Abd El-Hack, 2015) in laying hens. The use of bergamot oil (Citrus bergamia) in the diet of laying hens increased fatty acid composition (EPA and DHA) in the yolk (Bolukbasi et al., 2010). In our experiment, EFA dietary supplementation (containing powdered rosemary leave) did not affect serum antioxidant levels and egg yolk fatty acid composition.

Supplementing the diets at the levels of 0.5 and 1.0% with a similar EFA as that used in the study of Yasar and Yegen (2017) has a growth promoting effect in broiler chickens. In our trial, the egg production, egg weight and eggshell breaking strength were increased by addition of EFA into the diets of laying hens. No effects of EFA supplementations were observed on yolk's fatty acid composition and serum GPx, MDA, TAS and TOS levels.

These results were strongly supported by recent findings (Świątkiewicz et al., 2018). Dietary supplementation of hens at late laying period with sodium butyrate, probiotic bacteria, a blend of herb extracts or chitosan induced significant increases in laying rate, egg shell thickness and breaking strength with no effects on egg yolk fatty acid composition. In our study, a combined effect of several active agents from the EFA resulted in a cumulative beneficial effect on hen performance and egg quality. Moreover, the improvement of laying performance is greater in our study than that observed in the study of Świątkiewicz et al. (2018). Addition of antioxidant compounds including a source of herbal additive to the diet of hens during late laying period did not induce any changes in the body antioxidant level, but rather caused an increase in the egg yolk content of antioxidant (Loetscher et al. 2014). In our study, the blood serum levels of antioxidants were not influenced by the EFA addition, and it is likely that the antioxidant compounds available in the EFA was not sufficiently enough to induce such changes.

Conclusion

All the above indicated that the level of 1000 mg/kg EFA supplementation can be optimally be recommended for the diets of laying hens in order to enhance egg weight, egg production and eggshell breaking strength during late laying phase.

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