EFFECT OF DIETARY PROBIOTIC SUPPLEMENTATION OF LAYER HENS ON MORPHOLOGICAL AND FUNCTIONAL TRAITS OF EGGS

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ABSTRACT

Two lines of stock layers were included: line K (layers with white feathers used for obtaining autosexing hybrids as maternal line) and line L (Plymouth hens, used as maternal form for broiler chickens production). Sixty hens were included in each of experimental (whose ration was supplemented with probiotic) and control groups (non-supplemented). They were reared in 5 boxes with 4 nests in each, at a ratio of 12 hens per rooster in a nest.

Hens from line N received a standard compound feed according to their category. The diet of the experimental group was supplemented with 500 g probiotic/ton. The biologically active components of the bioproduct were: active microflora – *Lactobacillus delbrueckii subsp. bulgaricus* with total counts of 6.5×10^5, *Streptococus thermophilus* with total counts 3.4×10^8.

The addition of probiotic to the birds' feed led to a significant change of Live mass in hens of the meat-type L line at $p<0.001$. The percentage of hatchability from the set and fertile eggs was higher in the birds that had consumed probiotic of the N line – 89.92% and 96.67% at $p<0.01$ and $p<0.05$. The same indices for the L line were also higher in the experimental group, yet they were not statistically significant ($p\geq0.05$). From the results it could be concluded that the probiotic had no influence on the egg's size and morphological traits. The laying capacity in both groups of both lines did not differ significantly after the usage of probiotic. The addition of probiotic did not have a significant influence on the acidity of the egg content, as well as on the egg melange's ability to form emulsion, which is a very important functional trait.

Keywords: *probiotic, egg production, egg quality, layer, broiler breeder, productive trait.*

INTRODUCTION

The full prohibition on the usage of nutrient antibiotics to stimulate productivity in poultry has raised the interest towards the application of microbial products in feeds, which could substitute the antibiotics' effects.

Probiotics are one of suitable alternatives.

Unlike antibiotics, probiotics are living microorganisms, which have the ability to survive and multiply in the digestive tract, having an overall positive effect on the animal's body **(Dayle and Moy,** 2004**)**. They contain cells of yeasts, bacterial cultures or both, which stimulate microflora and are capable of altering the intestinal environment in order to improve overall health and the nutritional effect **(Dierck, N.,** 1989**).**

The addition of probiotics to the animals' feed is preferable because it does not produce any harmful effects on the end consumers **(Onifade et al.,** 1999**)**. **Chumpawadee et al., (**2009**)** have pointed out that their inclusion in hens' feed is beneficial during the periods of nutritional or other type of stress, yet has minimal effect on productivity and egg quality under normal environmental conditions.

According to **Yörük, M., et al. (**2004**)** when a probiotic is added to the feed of layer hens towards the end of the egg-laying period, higher egg productivity could be achieved without any detectable change in the eggs' quality. The authors also established linear reduction in mortality and feed consumption per unit of production. **Daneshyar et al. (**2007**)** have reported that the addition of different amounts of probiotic to the rations of broiler breeders did not have a noticeable effect on the percentage of the shell as a part of the egg's mass, its strength, relative weight, and Haugh units.

A number of authors believe that using probiotics in layer hens improved egg production and feed consumption per unit of production **(Nahashon et al.,** 1996a; **Mohan et al.,** 1995**; Abdulrahim et al.,** 1996**)**, feed consumption, **(Nahashon et al.,** 1994a**)** and egg mass **(Nahashon et al.,** 1996a**, Chumpawadee et al.,** 2009**).**

Due to contradictory data in the available literature, we aimed to establish the effect of probiotic addition in the rations of broiler breeders on the productive traits, as well as some morphological and functional qualities of the egg.

MATERIAL AND METHODS

The experiment was carried out between December 2010 and April 2011 with sexually mature birds at the Institute of Agriculture – Stara Zagora. Two lines of stock layers were included: line K (layers with white feathers used for obtaining autosexing hybrids as maternal line) and line L (Plymouth hens, used as maternal form for broiler chickens production). Sixty hens were included in each of experimental (whose ration was supplemented with probiotic) and control groups (non-supplemented). They were reared in 5 boxes with 4 nests in each, at a ratio of 12 hens per rooster in a nest.

Hens from line N received a standard compound feed according to their category. The diet of the experimental group was supplemented with 500 g probiotic/ton. The biologically active components of the bioproduct were: active microflora – *Lactobacillus delbrueckii subsp. bulgaricus* with total counts of 6.5×10^5, *Streptococus thermophilus* with total counts 3.4×10^8.

Hens from line L received a standard ration for the respective type and age. The diet of the experimental group was supplemented with probiotic at 500g/t.

The compound feed contained: metabolizable energy 3028.438 kcal/kg, crude protein 17.406%, crude fat 9.85%, fibre 6.183%, lysine 0.85%, methionine 0.419%, calcium 3.62%, phosphorus 0.81% and was recommended for this category of birds (**Todorov et al., 2007**).

I. Productive traits:

During the experimental period, the following parameters were controlled: live body weight (kg) at the age of 28 weeks and by the end of the trial; death rate (%),daily egg production (number of eggs), egg laying intensity (%) – on the basis of laid eggs for a determined time period, average egg mass (g) –at 2-week intervals; egg mass (g), feed conversion per egg laid (kg), feed conversion per 1 kg egg mass (kg); egg incubation traits – hatchability of total eggs set (%), hatchability of fertile eggs (%), embryonic death rate (%).

II. Egg morphology traits – evaluated at the age of 36 weeks. Thirty eggs were collected from each group for evaluation of:

1. Egg weight –with precision of 0.01g.
2. Albumen, yolk and eggshell weights – with precision of 0.01g
3. Thickness of eggshell with the eggshell membrane – determined at both poles and in the middle with a micrometre Ames 25EE with precision of 0.01 mm.
4. Haugh units – calculated by the formula

$HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$ by the method of Haugh (1937),

where H – the height of thick albumen (mm);

W – egg weight (g).

III. Functional traits, including:

1. pH of albumen, yolk and melange, determined by means of a pH meter *Pocket pH ad 110pH*
2. foaming properties of albumen – by a method of Sanovo, Odense, Denmark (1966)
3. albumen foam stability – by determination of the amount of exudate (ml) after 60 min;
4. emulsion properties, by mayonnaise test using the method for meat emulsion (Vassilev, 1986) modified by us.

Experimental results were processed with the EXCEL – ANOVA 2000 software.

Results and discussion

Table 1 shows the results on egg live mass. In the hens from the N line, of egg-laying type, no significant differences in the live weight of both groups could be observed from the beginning to the end of the experiment. Examining the influence of a probiotic preparation on layer hens, Sohail et al. (2002) did not prove its influence on the live weight of the experimental birds.

In hens from the L line, the experimental group with weight of 3400 g was significantly superior to the control group with live mass of 3138.98 g towards the end of the period ($p<0.001$). The addition of probiotic to the birds' feed led to a significant change of that trait.

Table 1 – Live mass of layer hens

Live mass (g)	At beginning of experiment		At end of experiment	
	control	experimental	control	experimental
line L	3082.98±52.31	3007.63±48.35	3138.98±40.45	3400±63.30 ***
line N	1416.67±9.18	1414.17±9.88	2061.02±23.56	2076.27±24.93

***at p<0.001

The reproductive traits of hens from both lines at the end of the probiotic addition to the feed are presented in Table 2.

Table 2 – Incubation qualities of eggs

line	Eggs set	Unfertilized	Fertilized	% fertilized	Embryonic death % early period	Embryonic death % Middle period	Embryonic death % Last period	% hatchability of eggs set	% hatchability of fertile eggs.
L control	181	13	168	92.75±1.27	1.71±0.48	0.6±0.68	4.17±0.57	87.22±1.63	94.03±0.58
L experimental	126	7	119	94.44±0.79	0.6±0.23	0	3,13±0,82	88,88±0.93	94.12±0.67
N control	130	9	121	93.08±1.13	0.82±0.03	3.31±0.64	1.65±0.82	87.69±0.73	94.21±0.57
N experimental	129	9	120	93.02±0.87	0	3.33±0.51	0	89.92±0.82*	96.67±0.67**

**at p<0.01 *at p<0.05

In the L line, the fertilized- experimental eggs were 94.44% that was higher than the rate in the control group (92.75%), yet the difference was insignificant ($p\geq0.05$).

In the N line, there were no significant differences in this trait. According to Kitanov I., et al, 2003, the reproductive traits fertility and hatchability from eggs set and fertile eggs differed insignificantly by 0.64; 6.4 and 6.5 points or 0.77%, 1.09%, 7.58% respectively higher than in breeder hens for production of broilers that consumed, in the feed mix, 2 ml of "Ovocap."

The level of embryonic death was low in the hens of the N egg-laying line. For the L line, a lower value was measured in the experimental group, yet the difference was insignificant ($p\geq0.05$).

The percentage of hatchability from set and fertile eggs was higher in the birds of the N line supplemented with probiotic – 89.92% and 96.67% at $p<0.01$ and $p<0.05$.

The same traits for the L line were higher for the experimental group, yet they were not statistically significant ($p\geq0.05$).

The morphological traits of the eggs from both groups of the respective lines are presented in Table 3.

Table 3 – Morphological qualities of the eggs- (n=30)

Parameters	L line - control	L line - experimental	N line - control	N line - experimental
	$x \pm Sx$	$x \pm Sx$	$x \pm Sx$	$x \pm Sx$
1. Egg weight, g	65.2±0.79	64.57±1.04	62.2±0.98***	57.56±0.76
2. Shell weight, g	6.86±0.18	6.47±0.17	6.72±0.14	6.26±0.12
3. Yolk weight, g	18.73±0.23	18.8±0.25	16.10±0.26***	14.73±0.28
4. Albumen weight, g of egg weight	38.83±0.72	39.03±0.75	38.41±0.86***	34.13±0.65
5. Haugh units	87.37±1.17	84.67±1.13	86.72±1.14	91.13±0.75**
6. Shell thickness, mm	0.29±0.005	0.29±0.006	0.35±0.006***	0.32±0.005

*** at p<0.001
** at p<0.01

From the 30 eggs randomly chosen for determining some of the basic morphological traits at the period's end, the differences between the indices were in favour of the control group for the L line, yet they were not significant (p≥0.05). In the N line, the weight of the eggs, the weight of the yolk and the albumen, as well as the shell thickness were significantly higher as compared to the control group (p<0.001), which was probably due to the random selection of eggs. But when the values of units Hough noticed conflicting results - in line L are mathematically unproven and at line N - reliable in experimental group at p<0.01.

From the results, it could be concluded that the probiotic had no effect on egg size and other relevant indicators. Similar were the reports by **Mahdavi et al.** (**2005**), who examined the effect of a probiotic preparation added to the feed of layer hens and reported an insignificant influence on the egg weight, laying capacity, the conversion of feed and the qualities of the eggs, along with significant differences in the eggs' cholesterol content.

In support of this statement, **Chen and Chen** (**2003**) reported that using probiotics in the hens' feed increased egg production but did not influence the mean weekly weight of the eggs and their qualities.

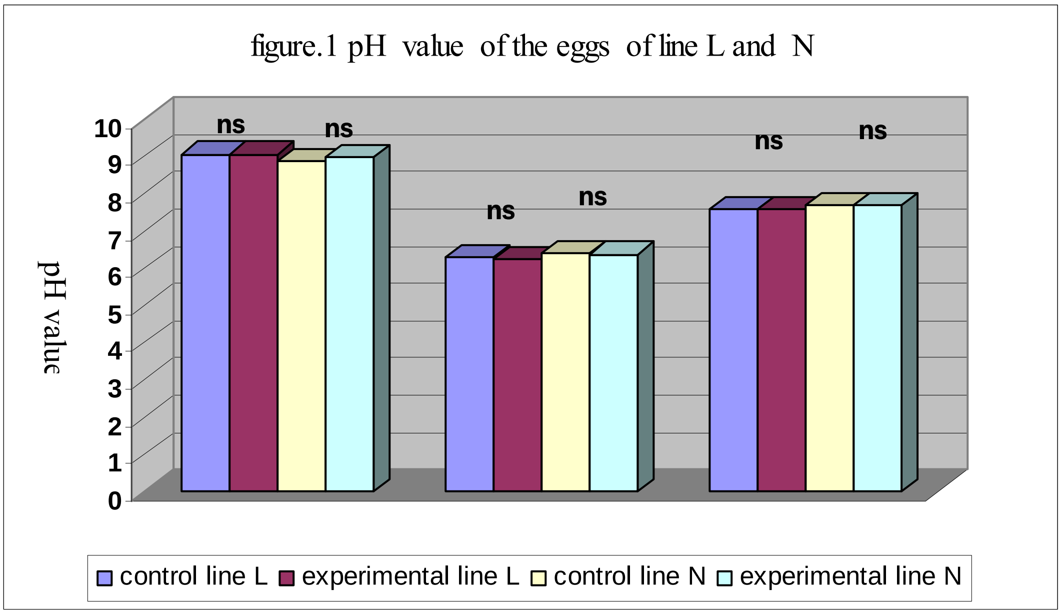
Table 4 presents the results on the hens' egg productivity from the control and experimental groups throughout the entire period. The laying capacity for both groups of both lines did not differ significantly after the addition of probiotic. In the L line, the experimental group produced 60.9 eggs and the control group – 59.14. In the N line, there was a decrease in laying for the experimental group by 5.69 eggs, yet the difference was not significant (p≥0.05). The usage of the probiotic did not influence the egg mass in both lines. For the L line, it remained 63.15 g, whereas in the N line there was a insignificant decrease of the trait from 59.48 g to 58.38 g. The feed conversion in control egg-laying type hens was lower at p<0.05. The results of our studies did not differ from those of **Kurtoglu et al.** (2004), who did not find any significant variations between the experimental and control groups of hens' feed consumption, egg weight, live weight, and yolk weight.

Table 4 –Egg productivity of hens

Indicators	L line		N line	
	Control	test	control	test
Laying capacity (number of eggs)	59.14±1,83	60.9±5.15	109.92±3.65	104.23±1.03
Laying intensity (%)	57.83±1.21	57.76±4.01	69.83±0.93	69.33±4.39
Mean egg weight (g)	63.96±0.45	63.15±0.71	59.48±0.92	58.38±0.69
Age of beginning of lay (days)	231±3.79	230.6±2.99	181.5±2.5	185.25±4.55
Feed conversion efficiency per egg (kg)	0.228±0.008	0.243±0.032	0.142±0.04	0.152±0.001*

*at $p<0.05$

Figure 1 shows the pH values of the albumen, yolk and melange in experimental eggs. Evidently, the differences between the control and experimental groups for both lines were insignificant, which proved that the probiotic did not influence the acidity of the egg contents.



The egg's albumen and yolk are colloid systems, capable of produce gel with a high dispersed phase volume. The colloid properties of a system are related to lipoproteins, which are able to produce gel.

According to Bernardi G. and W. Cook (1960), ovalbumin is the primary fraction, which comprises about ¾ of all proteins contained in the egg's albumen, whereas the ovovitellin makes up 4/5 of the yolk's protein content and consists of phosphoproteins. Thus, ovalbumin and ovovitellin make up the major part of the egg's protein mass and impart its emulsion properties.

The eggs' property to form emulsion has been established after performing a mayonnaise test with a ratio of melange/sunflower oil of 1/3, without the addition of preservatives. In both lines of control and experimental hens, a volume of 100 cm^3 was measured. The emulsions were stable after centrifugation for 10 minutes at 3000 rpm either immediately or after 24 hours.

This result gave us reason to assume that the experimental probiotic had no influence on the emulsion qualities of the egg content.

CONCLUSIONS

1. The addition of probiotic to the birds' feed led to a significant change of Live mass in hens of the meat-type L line at $p<0.001$.

2. The percentage of hatchability from the set and fertile eggs was higher in the birds that had consumed probiotic of the N line – 89.92% and 96.67% at $p<0.01$ and $p<0.05$. The same indices for the L line were also higher in the experimental group, yet they were not statistically significant ($p\geq0.05$).

3. From the results it could be concluded that the probiotic had no influence on the egg's size and morphological traits.

4. The laying capacity in both groups of both lines did not differ significantly after the usage of probiotic.

5. The addition of probiotic did not have a significant influence on the acidity of the egg content, as well as on the egg melange's ability to form emulsion, which is a very important functional trait.

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