The Effect of Probiotic and Humic Acids on Internal and External Egg Quality of Japanese Quails

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Abstract
A research was carried out to determine effect of probiotic and humic acids on external and internal egg quality parameters of Japanese quail housed in 3-floor cage technology. A total of 60 animals were divided into 3 groups. In the control group (n=20) birds fed on basal diet without any additive. Japanese quails in experimental group no. 1 (n=20) were received addition of probiotics preparation in a single dose of 1 $g.kg^{-1}$ of feed mixture. In the experimental group no. 2 (n=20) received a preparation of humic acids in a single dose of 3 $g.kg^{-1}$ of feed mixture. Feed mixture contained 11.7 MJ ME and 200.0 g crude protein. Feed and water were given ad libitum. We recorded statistically no significant differences (P>0.05) among groups in egg weight, egg shape index, eggshell weight, albumen weight, albumen percentage, albumen weight, Haugh units, yolk index and yolk colour. The yolk weight and yolk percentage were significantly lower (P≤0.05) in the group with the application of humic acids in feed mixture compared with the control group and the group with the addition of probiotics to feed. The parameters of eggshell quality (percentage, strength, thickness) were recorded significantly higher values (P≤0.05) in both experimental groups compared to the control.

Keywords: Japanese quail, egg, probiotic, humic acids, egg quality.

1. Introduction

The quality of eggs of different bird species is conditioned by many genetic and environmental factors [1], among others by genotype of birds [2-5], their age and stage of laying period [6-8], feeding methods [9-12], housing system and prevention programs [13-15], as well as environmental conditions of rearing, such as temperature and relative humidity, as well as the CO_2 content in the room [16,17], methods of storing eggs [18] and conditions of their distribution.

Probiotics are defined as live microbial food supplements, which beneficially influence on poultry health and performance [19]. Probiotics in poultry maintaining normal intestinal microflora by competitive exclusion and antagonism [20,21[, alter metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity

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and ammonia production [22,23], improve feed intake and stimulate the immune system [24-26].
Humates, originated from decomposed plants in the soil, have a very complex structure with molecular weight ranging from 5.000 to 200.000. Humates are composed of humic, ulmic and fulvic acids. Humic acids have ingredients of carbohydrates, amino acids and fenolic compounds [27]. In recent years the interest in the use of humic substances in animal husbandry has increased. Many authors in their studies observed an improvement in growth and feed conversion, and reduction of animal mortality after addition of humic substances into feedstuff [28-33]. Humid acid based mixtures have the potential to be an alternative to antibiotic growth promoters in broiler diets [34].
The objective of this study was to determine the effect of application of probiotic and humate on some parameters of internal and external egg quality of Japanese quail.

2. Materials and methods

Japanese quails were kept at the Research Institute of Animal Production in Nitra in cage technology by a proportion of 4 animals per cage of a 0.12 m^2 area.
Birds were divided to three groups. Group fed on basal diet without any additives served as control (n=20). Probiotic females (n=20) received an addition of a probiotics preparation in a single dose of 1 g kg^{-1} of feed mixture. The probiotic preparation on the basis of *Bacillus subtilis* (min. 1×10^7 cfu g^{-1}) and *Lactobacillus paracasei* (min. 1×10^7 cfu g^{-1}), glucose and lac desadipatum siccatum was purchased from Bioveta a.s. (Ivanovice na Hané, Czech Republic). Humate females (n=20) received a preparation of humic acids in a single dose of 3 g kg^{-1} of feed mixture. The humate preparation contained a minimum of 62% humic acids in the dry and was purchased from Humac s.r.o. (Košice, Slovak Republic). During the egg production period, Japanese quails were fed ad libitum commercial feed mixture for laying hens and quails (Tekro, Dvory nad Žitavou, Slovak Republic). Nutritional value of diets is shown in Table 1.
All animals were kept under standard environmental conditions in a thermoneutral hall (temperature 21±2°C with relative humidity 64±2%) during the whole experiment. Microclimate conditions were continually monitored using an electronic recorder (Hivus s.r.o., Žilina, Slovak Republic). The whole experiment lasted 210 days (7 months).
The analyse of 630 Japanese quail eggs (210 egg from each group) was performed in the laboratory of the Department of Small Animal Science of Slovak University of Agriculture in Nitra.
Egg weight was individually determined to 0.01g accuracy using a laboratory scale Owa Labor (VEB Wägetechnik Rapido, Germany). Egg length (along the longitudinal axis) and egg width (along the equatorial axis) were measured with a micrometer. Egg shape index was calculated as the ratio of egg width to length (%) by the method of [35].
After the eggs were broken, egg shells were washed with water and dried in order to clean the remaining albumen. Following this procedure, eggshell weight (with membrane) was measured using a laboratory scale Owa Labor (VEB Wägetechnik Rapido, Germany) and the percentage proportion of the eggshell in the egg was determined. Eggshell thickness (with membrane) was measured at three points: the blunt end, equator and the sharp end of each egg. Average eggshell thickness was obtained from the average values of these three parts. The egg shell strength was determined manually using an Egg Crusher device (VEIT Electronics, Czech Republic).
The albumen weight was calculated from the difference between the egg weight, and the yolk and shell weight and the percentage proportion of the albumen in the egg was determined. Albumen index (%) was determined by the method of [36] on the basis of the ratio of the thick albumen height (mm) measurement taken with a micrometer to the average of width (mm) and length (mm) of this albumen with 0.01mm accuracy. Haugh unit was calculated according to the procedure of [37].
Yolk weight with 0.01 g accuracy was determined using the laboratory scale Owa Labor (VEB Wägetechnik Rapido, Germany) and its percentage proportion was calculated. Yolk index (%) was measured on the basis of the ratio of the yolk height (mm) to the yolk width (mm) by the method of [38] using micrometer with 0.01mm accuracy. Yolk colour was determined with the

scale of Hoffman La Roche (Hoffman–La Roche, Switzerland).

The evaluated variables were submitted to analysis of variance using by JASP 0.8.6 software [39]. Means were compared as per Duncan's Multiple Range Test [40].

Table 1. Nutritional value in 1 kg complete feed mixture

Nutrient	Unit	
Crude protein	$g.kg^{-1}$	min. 200.00
Metabolic energy	$MJ.kg^{-1}$	min. 11.70
Lysine	$g.kg^{-1}$	min. 6.00
Methionine and Cysteine	$g.kg^{-1}$	min. 1.20
- from that Methionine	$g.kg^{-1}$	min. 3.50
Calcium	$g.kg^{-1}$	min. 35.00
Phosphorus	$g.kg^{-1}$	min. 5.00
Sodium	$g.kg^{-1}$	min. 1.60
Cooper	$mg.kg^{-1}$	min. 6.00
Zinc	$mg.kg^{-1}$	min. 40.00
Manganese	$mg.kg^{-1}$	min. 60.00
Iron	$mg.kg^{-1}$	min. 40.00
Vitamin A	$IU.kg^{-1}$	min. 15 000
Vitamin D3	$IU.kg^{-1}$	min. 2 000

3. Results and discussion

The effect of probiotic and humic acids on the egg parameters is presented in Table 2. We recorded that addition of tested feed additives had no significant effect (P>0.05) on egg weight of Japanese qual. Similar results about statistically no significant effect of probiotic on egg weight of hens observed [41,42]. In contrast, [43] noted that addition of probiotic with *Lactobacillus* significantly increased egg weight. Our results are no consistent with those reported by [44] who showed that the dietary humic acid at doses of 30 and 60 $g.t^{-1}$ feed can be used to improve egg weight. Equally, [45] indicated that the dietary humic substances at 5 or 10 % improved egg weights. Egg weight for hens fed diet containing humic acids at 0.1, 0.2 or 0.3% were significantly improved compared with the control hens [46]. Sopoliga et al. (2016) [47] did not confirm a positive impact of dietary humic substances addition at a level of 0.5% on egg weight of pheasant laying hens.

It was observed that there were no significant differences (P>0.05) among groups with respect to egg shape index. Also, humic substances did not affect egg shape index [48].

The eggshell percentage, eggshell strength and eggshell thickness significantly (P<0.05) increased for Japanese quails with addition of probiotic and humic acid compared with control group (Table 3). Whereas, no significant differences (P>0.05) were noticed among groups in eggshell weight.

Panda et al. (2003) [49] recorded that eggshell weight was significantly higher in the experimental group with probiotic.

A statistically significant difference (P<0.05) among the groups was recorded in the eggshell proportion which does not correspond to the results of [50], who noted only slight differences in this indicator.

The significantly higher eggshell strength observed in our study in the probiotic experimental group is not consistent with [44].

The significant higher effect of the probiotic on the eggshell thickness in accordance with our findings was noted by [50,51]. The beneficial effect of probiotics on eggshell quality has been reported by [52,53]. .

Our results approach with those reported by [45] who indicated that the eggshell strength as indicator of shell thickness was increased for hen fed diets with humic substances compared with the control group. While, [44] found that there were no changes in egg shell thickness and eggshell strength in hens supplemented with humic acid.

As shown Table 4, the yolk weight in our experiment was statistically no significant (P>0.05) affected by the addition of a probiotic to drinking water.

In our experiment, there was no statistically significant difference (P>0.05) between control and probiotics in yolk percentage. Similar conclusions were reached by [50]. Hong et al. (2002) [51] reported an increase in the yolk index with an increasing proportion of probiotics. Panda et al. (2003) [49] did not observe positive effects of the addition of probiotics in yolk quality in their experiment. In contrast, [54] noted statistically significant differences in yolk index.

Application humic acid statistically significant decrease (P<0.05) yolk weight and yolk percentage in comparison with control. Similarly, [53] observed significantly decrease of yolk percentage in hens fed humic acid compared with control group.

The values of yolk colour in the control and experimental groups were similar and differences among control and experimental groups were statistically no significant (P>0.05). Our results are consistent with the findings of [55]. However, [56] recorded a beneficial effect of probiotics and [51] noted improve the yolk colour for addition of probiotics.

In present study we noted statistically no significant differences (P>0.05) among groups in albumen quality (Table 5). Our results in Haugh unit are equally to [50,54,55,58,59] for probiotic Also, [48] recorded, that humic substances did not affect on Haugh unit.

Table 2. Effect of probiotic and humic acids on egg parameters

Parameter	Control	Probiotic	Humic acids
Egg weight (g)	12.31±2.65	12.39±2.89	12.33±2.71
Egg shape index (%)	75.87±0.62	75.97±0.64	75.78±0.65

Values shown are mean±SD (standard deviation)
[a,b] means in a row with different superscript differ significantly

Table 3. Effect of probiotic and humic acids on eggshell parameters

	Control	Probiotic	Humic acids
Eggshell weight (g)	1.06±0.23	1.11±0.27	1.10±0.25
Eggshell percentage (%)	8.71±1.78	8.98±1.88^a	8.96±1.85^b
Eggshell thickness (μm)	253.28±30.21	265.87±32.98^a	264.11±33.08^b
Eggshell strength (N.cm^{-2})	6.71±1.28	6.77±1.32^a	6.72±1.27^b

Values shown are mean±SD (standard deviation)
[a,b] means in a row with different superscript differ significantly

Table 4. Effect of probiotic and humic acids on yolk parameters

	Control	Probiotic	Humic acids
Yolk weight (g)	4.12±0.59^a	4.16±0.63^b	4.07±0.52
Yolk percentage (%)	33.78±1.56^a	33.63±1.62^b	33.01±1.49
Yolk index (%)	43.73±0.64	44.09±0.83	43.68±0.61
Yolk colour (°HLR)	6.89±0.72	6.82±0.75	6.78±0.69

Values shown are mean±SD (standard deviation)
[a,b] means in a row with different superscript differ significantly

Table 5. Effect of probiotic and humic acids on albumen parameters

	Control	Probiotic	Humic acids
Albumen weight (g)	7.03±0.88	7.12±0.92	7.16±0.97
Albumen percentage (%)	57.58±2.11	57.47±2.08	58.07±2.23
Albumen index (%)	10.89±0.22	10.91±0.28	10.82±0.25
Haugh Unit (%)	88.86±0.82	89.21±0.91	89.16±0.89

Values shown are mean±SD (standard deviation)
[a,b] means in a row with different superscript differ significantly

4. Conclusions

In this study, the effects of supplementation of humate and probiotic most significantly demonstrated in eggshell parameters. In albumen characteristics was effect of tested feed additives had no consistent effects. In yolk parameters, addition of humic acids had a negative impact on yolk weight and yolk percentage.

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