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Original scientific paper

THE EFFECT OF SINGLE AND COMBINED USE OF PROBIOTIC AND HUMATE ON FATTENING PERFORMANCE, CARCASS CHARACTERISTICS AND INTERNAL ORGANS OF BROILER CHICKENS

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A b s t r a c t: The aim of this study was to evaluate the both single and combined effects of probiotic and humate on performance and carcass characteristics of broiler chickens. Totally 200 one-day old broiler chickens Ross 308 were divided into one control group and three experimental groups. The control group (n = 50) was without probiotic and humate, experimental groups with 3.7 ml on 1 litre of drinking water (Group P, n = 50), 50 g per 10 kg of feed (Group H, n = 50) and 3.7 ml probiotic per litre of drinking water + 50 g humate per 10 kg of feed (Group P + H, n = 50), respectively. Application of probiotic and humate both single and combined statistically significant increased ($p < 0.05$) final body weight and reduced feed consumption ($p < 0.05$). From carcass characteristics, application of probiotic and probiotic and humate combination statistically significant decreased ($p < 0.05$) abdominal fat weight. Supplementation of single and combined use of probiotic and humate no significantly affected ($p > 0.05$) breast and thighs percentages and carcass yield. The caecum, small intestine and large intestine proportions significantly ($p < 0.05$) increased in chickens fed diets containing probiotic compared to control. For neck, crop, heart, liver, proventriculus, gizzard, pancreas and kidneys proportions among control and experimental groups we recorded no statistical differences ($p > 0.05$).

Key words: chicken; probiotic; humate; performance; carcass parameters

ВЛИЈАНИЕ НА ЕДИНИЧНА И КОМБИНИРАНА УПОТРЕБА НА ПРОБИОТИК И ХУМАТ ВРЗ ОСОБИНИТЕ НА ГОЕЊЕ, КАРАКТЕРИСТИКИ НА ТРУПОТ И ВНАТРЕШНИТЕ ОРГАНИ КАЈ БРОЈЛЕРСКИТЕ ПИЛИЊА

А п с т р а к т: Целта на ова истражување беше да се оценат единичните и комбинираните ефекти на пробиотик и хумат врз перформансите и особините на трупот кај бројлерските пилиња. Вкупно 200 еднодневни бројлерски пилиња од хибридот Ross 308 беа распоредени во една контролна и три експериментални групи. Контролната група (n = 50) беше без додаток на пробиотик и хумат, додека кај експерименталните групи беа додадени 3,7 ml пробиотик на литар вода за пиење (група P, n = 50), 50 g хумат на 10 kg храна (група H, n = 50), и 3,7 ml пробиотик на литар вода за пиење + 50 g хумат на 10 kg храна (група P + H, n = 50), соодветно. Примената на пробиотикот и хуматот, како единично така и комбинирано, статистички значајно ја зголемила ($p < 0,05$) финалната телесна тежина и ја намалила потрошувачката на храна ($p < 0,05$). Во однос на карактеристиките на трупот, примената на пробиотикот и комбинацијата на пробиотик и хумат статистички значајно ја намалиле ($p < 0,05$) содржината на абдоминалната маст. Единечната и комбинираната употреба на пробиотик и хуматот во исхраната имала незначајно влијание ($p > 0,05$) врз процентот на градното месо и копаните како и на приносот на трупот. Пропорциите на цекумот, тенките црева и дебелото црево значително ($p < 0,05$) се зголемиле кај бројлерите кои се хранеле со смеси кои содржат пробиотик во споредба со контролните. Пропорциите на вратот, гушата, срцето, црниот дроб, провентикулусот, желудникот, панкреасот и бубрезите помеѓу контролните и експерименталните групи не покажале статистички разлики ($p > 0.05$).

Клучни зборови: пилиња; пробиотик; хумат; перформанси; карактеристики на труп

1. INTRODUCTION

The European Union has banned the use of antibiotic feed additives due to their negative effect on human health (Immerseel et al., 2004). At present, several substances such as probiotics, organic acids, and oligosaccharides are used as yield enhancers in broiler production. These yield-enhancing substances can be used either alone or in combination with other substances (Ashayerizadeh et al., 2009).

Probiotics are one of the approaches that have a potential to reduce chances of infections in poultry and subsequent contamination of poultry products (Ahmad, 2006). Probiotics are defined as live bacteria-yeast cultures or biological products that are added to drinking water or feed to regulate the ecological balance of microflora in the digestive tract of animals. These substances prevent the harmful effects of potentially pathogenic microorganisms and allow animals to derive increased benefits from the feed (Dibner and Richards, 2005). The selection of bacteria such as *Lactobacillus, Pediococcus, Bacteroides, Bifidobacterium, Bacillus* and *Streptococcus*, for use as probiotics is based on assessment of their metabolic products and their potential to colonize specific sites (Lima et al., 2007). Several studies have shown that addition of probiotics to the diet of broiler and turkey leads to improve the performance (Vicente et al., 2007).

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 (Şahin et al., 2011). 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 (Eren et al., 2000; Kocabağli et al., 2002; Karaoglu et al., 2004; Ji et al., 2006; El-Husseiny et al., 2008; Šamudovská and Demeterová, 2010). Humic acid based mixtures have the potential to be an alternative to antibiotic growth promoters in broiler diets (Ceylan et al., 2003).

The objective of this study was to determine the effect of single and combined application of probiotic and humic acids preparations on productive parameters and carcass characteristics of Ross 308 broiler chickens.

MATERIAL AND METHODS

Broiler chickens were stabled in a 3-etage cage technology (MBD, Czech Republic) consisted of 18 cages with proportions 75 × 50 cm (0.375 m^2).

All of the chicks were reared under in closed hall with standard temperatures 33°C at chick level for 1 week, followed by a reduction of 2°C/week until the temperature reached 19°C at 6 week of age. Artificial light was provided during the over fattening period (23 h light : 1 h dark). Temperature was continually monitored using electronic recorder (Hivus s.r.o., Žilina, Slovak Republic).

Broiler chickens were fed commercial feed mixtures (PPD Prašice, Slovak Republic): starter (days 1 to 21), grower (days 22 to 35) and finisher (days 36 to 42). The nutritive values of the feed mixtures are presented in Table 1.

Table 1

Nutritive values of feed mixtures in experiment

Nutrient	Units	Starter	Grower	Finisher
Crude protein	%	min. 20.00	min. 18.30	min. 17.00
Fat	%	min. 4.80	min. 4.00	min. 6.00
Fibre	%	max. 4.00	max. 5.00	max. 5.00
Lysine	%	min. 1.20	min. 1.10	min. 0.90
Methionine	%	min. 0.52	min. 0.48	min. 0.45
Calcium	%	min. 0.80	min. 0.80	min. 0.55
Phosphorus	%	min. 0.55	min. 0.55	min. 0.50
Sodium	%	min. 0.12	min. 0.12	min. 0.12
Cupper	mg	min. 15.00	min. 15.00	min. 15.00
Zinc	mg	min. 80.00	min. 80.00	min. 80.00
Manganese	mg	min. 120.00	min. 70.00	min. 100.00
Iron	mg	min. 120.00	min. 100.00	min. 100.00
Iodine	mg	min. 0.90	min. 0.40	min. 0.40
Selenium	mg	min. 0.20	min. 0.10	min. 0.10
Vitamin A	I.U.	min. 12000	min. 10000	min. 10000
Vitamin D_3	I.U.	min. 5000	min. 5000	min. 5000
Vitamin E	mg	min. 60.00	min. 50.00	min. 50,00
Natrium salinomycinate	mg	60.00	60.00	–
Endox	mg	125.00	125.00	125.00

Totally 240 broiler chickens Ross 308 were divided into four groups (control – C, and experimental groups – E1, E2 and E3). Experimental chickens

of E1 group (n = 60) received a probiotic preparation (IPC s.r.o. Košice, Slovak Republic) in drinking water with concentration of 1 × 10^9 colony forming units (CFU) of *Lactobacillus fermentum CCM 7158* in 1 g of nutrient medium with supporting components maltodextrin and oligofructose (1% in preparation). Experimental chickens of E2 group (n = 60) received a humate (Humac s.r.o., Košice, Slovak Republic) with 3 g/kg feed mixture. Feed addition used in this study contained a minimum 62% humic acid and 9% fulvic acids in the dry. Experimental chickens of E3 group (n = 60) received a combination of probiotic preparation in drinking water with concentration of 1 × 10^9 colony forming units (CFU) of *Lactobacillus fermentum CCM 7158* in 1 g of nutrient medium and humate with 3 g/kg feed mixture. Quantization of drinking water and probiotic preparation is presented in Table 2. The control group of animals received feed mixture and drinking water without any additives. The fattening period lasted 42 days.

Table 2

Design of experimental intervention

Week of age	Total amount of drinking water per day (l)	Quantization of probiotic strain (g)	CFU in 1 ml of drinking water
1	2.50	6.60	2.64×10^6
2	3.50	6.60	1.90×10^6
3	4.60	3.70	8.04×10^5
4	6.70	3.70	5.52×10^5
5	8.60	3.70	4.30×10^5
6	10.60	3.70	3.49×10^5

During the experiment broiler chickens were weighted for individual body weight at day1, day7, day14, day21, day28, day35 and day42 of age and body weight gain were calculated as the difference between the final and initial chicken weight. Feed consumption and mortality were recorded at 42 days of fattening period.

At 42 days of fattening, representative 10 chickens with body weight similar to the mean were chosen from each group for slaughter weighed and subjected to a 12-hours feed withdrawal. After slaughter, carcasses were weighed and subjected to simplified dissection. Abdominal fat, breast and drumstick were collected and weighed. The organs development was measured by taking weight of the broilers after slaughtering. Neck, crop, heart, proventriculus, gizzard (empty gizzard), liver (without gall bladder), pancreas, caecum, kidney, small intestine and large intestine weights were recorded individually and their percentages in relation to live body weight were calculated. The results obtained were used to calculate dressing percentage and the percentage of carcass components.

Data were subjected to analysis of variance using one way ANOVA procedure of the statistical system at JASP 0.8.6 software (JASP, 2018). Differences between means were ranked by Duncan's multiple range test of significance level of 5% (Duncan, 1955).

RESULTS AND DISCUSSION

As shown in Table 3, supplementation with humate and probiotic was most evident in combined addition of both additives, equally significantly increased ($p < 0.05$) final body weight we recorded in treatments with single addition of probiotic and humate in comparison with control treatment.

Table 3

Effect of probiotic and humate on body weight of broiler chickens

Week of age	Control	Probiotic	Humate	Probiotic+Humate
1.	45.68 ± 2.78	46.21 ± 2.96	46.19 ± 2.84	45.97 ± 2.91
7.	113.07 ± 19.76	115.97 ± 21.61	115.26 ± 21.89	116.87 ± 22.05
14.	304.78 ± 48.68	312.41 ± 51.29	311.97 ± 49.84	312.84 ± 50.86
21.	611.43 ± 89.65	653.28 ± 92.57	652.71 ± 93.28	654.69 ± 93.28
28.	1126.67 ± 138.64	1214.83 ± 141.28^a	1211.79 ± 140.39^b	1229.93 ± 142.84^c
35.	1611.62 ± 198.34	1721.68 ± 202.24^a	1720.24 ± 203.95^b	1746.51 ± 205.18^c
42.	2074.18 ± 241.59	2196.24 ± 260.24^a	2189.59 ± 259.97^b	2218.34 ± 262.38^c

Legend: Values marked by letters (a, b, c) in one row describe significant differences ($p < 0.05$).

Our results agree with the work of Zulkifli et al. (2000), Lan et al. (2003), Kabir et al. (2004), Weis et al. (2010), Weis and Hrnčár (2013) and Hrnčár et al. (2014), who observed improvement of final body weight of broiler chickens at addition of probiotics. In contrast, they are opposite to those of Ergun et al. (2000), Gunal et al. (2006) and Mountzouris et al. (2007), who found that the use of probiotic products in the feed had no significant effect on body weight of broiler chickens. Probiotic provides nutrients, effectively stimulates the growth of beneficial microflora in the small and large intestines resulting in the better balance of bacterium population (Yusrizal and Chen, 2003; Midilli et al., 2008; Capcarová et al., 2010, 2011). The increased body weight gain of broiler chickens fed probiotic may be due to improvement in digestibility and availability of many nutrients such as proteins, fats and carbohydrates, as well as some mineral elements and vitamins (Burkholder et al., 2005). Ceylan et al. (2003) reported that supplementation level of 0.25% humate enhanced body weight gain of broilers and these results supported results of our study. But Kocabağlı et al. (2002), Karaoglu et al. (2004) and Yalcin et al. (2005) have reported that 0.1–0.25% humate additions did not affect body weight gain of broilers. On the contrary, supplementation of 0.5–2.5% humic acid in ration decreased body weight gain of broilers (Rath et al., 2006). This study is in accordance with the studies where humic acid had effect ($p < 0.05$) on body weight gains on broilers (Eren et al., 2000; Kocabağlı et al., 2002). The results of the studies on humate and probiotic addition to the broiler diets (Yalçın et al., 2003; Kaya and Tuncer, 2009) revealed similar results to ours.

Total feed consumption was not affected by addition of probiotic and humate. We noticed the lowest feed consumption for probiotic (1.89 kg), followed by the treatments probiotic+ humic acids (1.90 kg) and humate (1.92 kg). The highest feed consumption was detected in control (1.94 kg). The results obtained from this research are in agreement with Yu et al. (2007) who reported that probiotic inclusion did not significantly affects feed consumption. In contrast, some researchers found a positive effect of dietary probiotic supplementation (Samli et al., 2007; Baurhoo et al., 2009).

The mortality rate was improved in treatments probiotic+humate and humate (3.33%) in contrast with treatments probiotic and control (6.67%). Also Cmiljanić et al. (2001) proved a reduction of mortality rate due to the addition of probiotic in feeding of broiler chicken. It was reported that humic acids supplementation in ration decreased feed consumption of broilers and hens (Rath et al., 2006). On the contrary, Ceylan et al. (2003) have reported that humic acids supplementation in ration did not affect feed consumption of broilers and hens. Differences in feed consumption may have resulted from the differences in amount of added water. Also, Yasar et al. (2002) have reported that humic acids supplementation in drinking water of rats did not affect feed consumption. Humates promote growth by altering partitioning of nutrient metabolism (Kocabağli et al., 2002; El-Husseiny et al., 2008; Ozturk et al., 2012; Mirnawati and Marlida, 2013), and reducing mortality (Eren et al. 2000) and improving feed conversion efficiency (Eren et al., 2000).

Differences in breast and thighs of chicken from control and experimental treatments (Table 4) were not statistically significant ($p > 0.05$). Opposite tendency observed Kabir et al. (2004), who reported that addition of probiotic would increase of efficiency of thigh and breast. As shown in Table 4, there were no differences ($p > 0.05$) between groups on carcass yield of broiler chickens. Similar values of carcass yields in broiler chickens supplemented or not with probiotics were found by Pelicano et al. (2004) and Weis et al. (2010).

Table 4

Effect of probiotic and humate on carcass parameters of broiler chickens

	Control	Probiotic	Humate	Probiotic+Humate
Breast (%)	30.21±1.68	30.89±1.72	30.86±1.74	30.85±1.73
Thighs (%)	31.23±2.07	31.78±2.04	31.76±2.01	31.82±2.02
Abdominal fat (g)	45.78±4.05	41.29±3.28[a]	44.84±4.12	41.54±3.67[b]
Carcass yield (%)	75.41±2.11	75.56±2.22	75.55±2.18	75.59±2.17

Legend: Values marked by letters (a, b) in one row describe significant differences ($p < 0.05$).

The experimental treatments fed with probiotic and probiotic+humate had significantly lower weight of abdominal fat compared with control. Equally, Kalavathy et al. (2003) and Weis et al. (2010) observed significant reduction of the supplementation of probiotic on abdominal fat content of the chicken.

Broiler chickens fed diets containing probiotic had significantly increased ($p < 0.05$) caecum, small intestine, and large intestine proportions compared with control (Table 5). The neck, crop, heart, liver, proventriculus, gizzard, pancreas, and kidney proportions between control and experimental groups did not show statistical differences ($p > 0.05$).

Table 5

Effect of probiotic and humate on intestinal organs of broiler chickens (%)

Internal organ	Control	Probiotic	Humate	Probiotic+Humate
Neck	2.99 ±0.34	3.04 ±0.35	2.97 ±0.38	3.02 ±0.33
Crop	0.27 ±0.06	0.24 ±0.05	0.24 ±0.07	0.25 ±0.06
Heart	0.63 ±0.13	0.66 ±0.15	0.64 ±0.16	0.63 ±0.11
Liver	1.94 ±0.29	1.97 ±0.33	2.01 ±0.39	1.99 ±0.35
Proventriculus	0.34 ±0.11	0.36 ±0.09	0.35 ±0.09	0.37 ±0.08
Gizzard	0.96 ±0.15	0.94 ±0.14	0.95 ±0.12	0.97 ±0.14
Pancreas	0.13 ±0.06	0.15 ±0.05	0.16±0.05	0.15 ±0.07
Caecum	0.51 ±0.11	0.69 ±0.14[a]	0.62±0.12	0.63 ±0.11
Kidney	0.67 ±0.16	0.69 ±0.12	0.67 ±0.13	0.69 ±0.15
Small intestine	2.36 ±0.38	2.57 ±0.42[a]	2.34 ±0.37	2.34 ±0.33
Large intestine	0.17 ±0.07	0.24 ±0.09[a]	0.22 ±0.08	0.21 ±0.05

Legend: Values marked by letter (a) in one row describe significant differences ($p < 0.05$).

Such findings have been reported in the literature. For example, Olnood et al. (2015) found, that probiotics increased relative weight of the jejunum and ileum in 42 days old birds compared with controls. The weights of liver, pancreas, gizzard and duodenum were not affected by the treatments. Pedroso et al. (2003) added *Lactobacillus reuteri* and *Lactobacillus johnsonii* into drinking water and reported a significant increase in intestinal weight in broilers. The mechanism by which this occurs is not known as the effect of probiotics on organ weights in animals is equivocal. Thus, Guan et al. (2003) found that supplementation of broiler diets with lactobacilli did not affect the weight of the intestine. Ozturk et al. (2010) found that humic substances of tested doses (150 ppm, 300 ppm and 450 ppm) did not affect on weight of gizzard, heard and liver.

CONCLUSIONS

The results revealed that using probiotic and humate in single and combined use positively affects growth of chicken broilers, had significant effects on final body weight and decreased feed consumption. Addition of probiotic and humate has not affected body composition, carcass yield and weight of internal organs. Supplementation of probiotic and combination probiotic and humate are favourable to the consumers as broilers have less abdominal fat content. In conclusion, it can be stated, that probiotic and humate might be promising alternatives for antibiotics eliminate in broiler chicken production.

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