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EFFECT OF DIET SUPPLEMENTED WITH PROPOLIS EXTRACT AND PROBIOTIC ADDITIVES ON PERFORMANCE, CARCASS CHARACTERISTICS AND MEAT COMPOSITION OF BROILER CHICKENS

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ABSTRACT

The present research focused on the effects of propolis extract and probiotic preparation based on *Lactobacillus fermentum* (1 × 10^9 CFU per 1 g of bearing medium) on performance, carcass characteristics and meat composition of broiler chickens. The experiment was performed with 360 one day-old Ross 308 broiler chicks of mixed sex. The chicks were randomly allocated into 3 groups (n = 120 pcs chicks per group), namely, control (C) and experimental (E1, E2). Each group consisted of 3 replicated pens with 40 broiler chickens per pen. The experiment employed a randomized design, and dietary treatments were as follows: 1. basal diet with no supplementation as control (group C), 2. basal diet plus 400 mg propolis extract per 1 kg of feed mixture (group E1), 3. basal diet plus 3.3 g probiotic preparation added to drinking water (group E2). Besides, the groups were kept under the same conditions. Fattening period lasted for 42 days. Feed mixtures were produced without any antibiotic preparations and coccidiostats. As regards performance of broilers, all the investigated parameters were improved after addition of the supplements, especially after probiotic supplementation. However, neither propolis extract nor probiotic in diet of broiler chickens had any significant effect ($p \geq 0.05$) on performance. Meat composition was evaluated as proximate composition (dry matter, crude protein, fat and ash), cholesterol content and energy value in the most valuable parts of chicken meat (breast and thigh muscles). The statistically significant results ($p \leq 0.05$) were attained in fat, ash and cholesterol content, as well as energy value in both breast and thigh muscles after the propolis supplementation. To sum up, the present study demonstrated the promising potential of propolis extract and probiotic to enhance the performance, carcass characteristics and meat composition under conditions of the experiment with, however, statistical significance of results in a few parameters.

Keywords: performance; meat; chicken; propolis; probiotic

INTRODUCTION

Chickens are the most popular amongst different poultry species worldwide. Owing to their relatively low fat and cholesterol contents, chicken meat is considered a healthy animal food. Moreover, chicken continues to be the cheapest among all types of meat consumed in the world and its consumption is expected to increase by 34% by 2018 **(Umaya Suganthi, 2014; Petrová et al., 2015)**. Modern intensive chicken production has achieved phenomenal gains in the efficient and economical production of high quality and safe chicken meat. The use of feed additives has been an important part of achieving this success **(Hashemi et al., 2012)**.

For several decades, antibiotics have been widely used in the chicken diet **(Goodarzi and Nanekarani, 2014)**. However, the use of dietary antibiotics have resulted in controversial problems such as development of antibiotic resistant bacteria and drug residue in the final products which can be harmful to consumers **(Goodarzi et al., 2014)**. As a result, additives such as probiotics and natural substances such as propolis have received increased attention as possible antibiotic growth promoter substitutions in chicken diet **(Haščík et al., 2012; Daneshmand et al., 2015)**.

Propolis is a resinous material elaborated by bees, through the recollection of the exudates from different plant species **(Valenzuela-Barra et al., 2015)** and is used in construction and adaptation of their hives. It possesses many pharmacological activities, such as anti-inflammatory, antibiotic, antiviral and immunostimulant **(Fan et al., 2013)**.

In many studies conducted on propolis, many positive effects like increase in feed intake, body weight, flavonoid content, taste improvement, antioxidant and antimicrobial properties have been reported **(Tatlı Seven et al., 2008)**. The properties of propolis are based on its rich flavonoid, phenolic acid and terpenoid contents **(Seven et al., 2012)**.

An alternative approach to subtherapeutic antibiotics in chicken diet is also the use of probiotic microorganisms **(Alkhalf et al., 2010)**. Probiotics are live, non-pathogenic bacteria that contribute to the health and balance of the intestinal tract **(Giannenas et al., 2012)**. The most important advantage of a probiotic is that it neither has any residues in animal production nor exerts any antibiotic resistance by consumption **(Alkhalf et al., 2010)**. Several studies showed that dietary supplementation of lactic acid bacteria (e.g. *Lactobacillus*) improve the performance and feed conversion **(Taklimi et al., 2012; Bai et al., 2013)**, stimulate immune response and increase bone strength of broiler chickens. The enhanced growth with probiotics may be partly attributed to the colonisation of the gastrointestinal tract of the chicks, which improved the digestion of essential nutrients **(Khaksefidi and Rahimi, 2005)**.

This study was designed to investigate the effects of dietary addition of propolis extract and probiotic preparation based on *Lactobacillus fermentum* on performance, carcass characteristics and meat composition of Ross 308 broiler chickens.

MATERIAL AND METHODOLOGY

Chickens and dietary treatments

The experiment was carried out in test poultry station of Slovak University of Agriculture in Nitra. A total of 360 one day-old broiler chicks of mixed sex (Ross 308) were randomly divided into 3 groups, namely, control (C) and experimental (E1, E2). Each group consisted of 3 replicated pens with 40 broiler chickens per pen. The experiment employed a randomized design, and dietary treatments were as follows: 1. basal diet as control (group C), 2. basal diet plus 400 mg propolis extract per 1 kg of feed mixture (group E1), 3. basal diet plus 3.3 g probiotic preparation added to drinking water (group E2). Besides, the groups were kept under the same conditions.

The experiment lasted for 42 days. The broiler chickens were reared on breed litter (wood shavings), in a temperature-controlled room; ambient temperature in test poultry station was maintained at 33 °C during the first week and gradually decreased by 2 °C, and finally fixed at 19 °C thereafter. Throughout the entire experimental period, the chickens had *ad libitum* access to feed and water, and were kept under constant light regime.

Table 1 lists the basal diet formulated according to nutrient requirements of broilers. The broiler chickens were fed a starter diet from 0 to 21st day and grower diet from 22nd to 42nd day. The feed mixtures both starter and grower were produced without any antibiotics and coccidiostats.

Propolis had origin in the Slovak Republic. The extract was prepared from minced propolis in the conditions of the 80% ethanol in the 500 cm^3 flasks, according to **Krell (1996)**. Determination of phenolic compounds, namely the phenolic acids (caffeic acid, *p*-coumaric acid, ferulic acid, cinnamic acid) and flavonoids (routines, quercetin, kaempferol, apigenin, tectochrysin) in propolis extract (Table 2) was performed using an Agilent 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a degasser, an autosampler and a diode array detector (DAD).

In the experiment, the probiotic preparation based on *Lactobacillus fermentum* (1 × 10^9 CFU per 1 g of bearing medium) was used.

Slaughter and measurements

At 42 days of age, chickens were weighed and slaughtered at the experimental slaughterhouse of Slovak University of Agriculture in Nitra.

After evisceration, the carcasses were kept at approximately 18 °C for 1 h *post mortem* and thereafter longitudinally divided into two parts. After that, the half-carcasses and giblets were weighed and stored at 4 °C until 24 h *post mortem*. The right half-carcasses were used in order to determinate the parameters as described below, whereas the left half-carcasses were assigned to different analysis. All the weight measurements were performed using the precision balance Kern 440 (Kern & Sohn, Germany) with accuracy of 0.01 g. The carcass yield was calculated by dividing carcass weight with giblets and abdominal fat weight by live body weight.

The chemical analysis of chicken meat (breast muscle without skin, thigh muscle with skin and subcutaneous fat) was performed using an Infratec 1265 Meat Analyzer. The cholesterol content of chicken meat was determined by spectrophotometric method according to **Horňáková et al., (1974)**. The energy value (kJ/100 g) was calculated through the conversion factors for fat and protein **(Strmiska et al., 1988)**.

Statistical analysis

The data processing was performed using a statistical program Statgraphics Plus Version 5.1 (AV Trading Umex, Dresden, Germany). For the determination of significant difference between the tested groups, analysis of variance (ANOVA) was used.

RESULTS AND DISCUSSION

The effects of propolis and probiotic supplementation on performance and carcass characteristics of Ross 308 broiler chickens are shown in Table 3. Live body weight of broilers did not differ statistically between the control and experimental groups ($p \geq 0.05$). Similarly, no differences ($p \geq 0.05$) were found between the groups in carcass weight, giblets weight and carcass yield.

Yet, effect of the supplementation has shown to be favourable since the chickens fed diet containing the propolis extract (2316.9 g) and probiotic preparation (2335 g) had higher live body weight than control chickens (2270.2 g).

The results of the study for performance and carcass characteristics of broiler chickens are in general agreement to those of previous studies where the inclusion of propolis in chicken diet also resulted in slight effect on meat performance.

Tatlı Seven et al., (2008) found higher body weight of chickens fed a diet supplemented with 0.5, 1 and 3 g propolis extract per 1 kg of feed mixture (1975 – 2010 g) than that in control (1940 g).

Shalmany and Shivazad (2006) showed that propolis extract in levels 200 and 250 mg.kg^{-1} has positive effect on growth performance of chickens due to improved weight gain and feed efficiency compared with chickens fed a basal diet.

Table 1 Composition of basal diet and nutrient content.

Ingredients (%)	Starter (HYD-01) (day of age 1 – 21)	Grower (HYD-02) (day of age 22 – 42)
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48% N)	21.30	18.70
Fish meal (71% N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysine	0.05	0.07
Methionine	0.15	0.22
Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 0.5%*	0.50	0.50
	Nutrient content (g.kg^{-1})	
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
Linoleic acid	13.51	14.19
ME_N (MJ.kg^{-1})	12.02	12.03

* active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 20 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; D-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 20 000 mg; folic acid 400 mg; biotin 40 mg; kobalamin 8.0 mg; choline 100 000 mg; betaine 50 000 mg; Mn 20 000 mg; Zn 16 000 mg; Fe 14 000 mg; Cu 2 400 mg; Co 80 mg; I 200 mg; Se 50 mg.

Positive effects of propolis were also observed in the study of **Biavatti et al., (2003)**, where effects of propolis extract, the *Alternanthera brasiliana* extract and lindseed oil as alternative feed additives were evaluated. The researches have suggested the additives in diet of broiler chickens due to improved broiler performance in the same way (similar body weight ($p \geq 0.05$) among the treatments that was higher than that in the control).

In another study **(Ziaran et al., 2005)**, body weight of chickens (47 day-old) fed a diet containing different levels of propolis (oil extract) was not affected when compared to those fed a diet containing no supplement (1916.64 – 1935.67 g vs. 1912.08 g).

Similar to the present findings, **Haščík et al., (2014)** demonstrated that propolis extract (200, 300, 400 mg.kg^{-1}) added in feed mixture increased the body weight of broiler chickens (2354.6 – 2382.9 g). However, no major effects on chicken growth performance were observed (2272.89 g in control group).

In contrast, **Açıkgöz et al., (2005)** reported significant decrease in body weight of male broilers after propolis supplementation (powder). The body weight of chickens fed diet containing propolis powder ranged from 2061 to 2229 g compared with that in control group (2302 g). In the study, pine originated propolis, which is characterized by strict genuine odour, volatile compounds and a bitter taste, was used. Because of these specific characteristics, broilers might reject the feed mixture that results in adverse effects on growth performance.

In the study of **Daneshmand et al., (2015)**, the body weight of broiler chickens (42 day-old) fed a diet containing 200 mg.kg^{-1} propolis extract (2395 g) was also lower compared with that in the control (2433 g). On the contrary, probiotic preparation (0.45 g.kg^{-1} of feed mixture) containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum* and

Table 2 Concentration of analysed phenolic compounds in propolis extract.

Compound	RT[1] (min)	Concentration (mg.g^{-1})
Caffeic acid	8.48	4.976 ±2.049
p-Coumaric acid	12.83	9.826 ±8.232
Ferulic acid	14.00	7.436 ±6.710
Cinnamic acid	26.47	0.367 ±0.182
Routines	22.33	4.578 ±1.714
Quercetin	29.59	2.963 ±0.762
Kaempferol	32.93	2.503 ±0.502
Apigenin	33.69	3.970 ±2.181
Tectochrysin	37.00	7.523 ±3.959

[1]RT – retention time

Enterococcus faecium used in the same study increased the body weight of experimental chickens (2527 g). However, there was no significant increase ($p \geq 0.05$). Moreover, there was investigated the effects of propolis in combination with the probiotics (0.20 and 0.45 $g.kg^{-1}$ of feed mixture, respectively). Although the combination did not significantly affect performance, the body weight of broiler chickens receiving a combination of these additives was higher than that in control. It may reflect synergetic and complementary effects between the additives in diet of broiler chickens.

As far as the probiotics are concerned, there is considerable variation in published studies that evaluate the effect of probiotic strains on performance of broiler chickens.

There are conflicting reports on the effects of application of probiotics because the response of broiler chickens to probiotics can be affected by different factors such as the duration and method of probiotic feeding, dose and nature of the administered strains and their persistence, variation in the physiological state of the chicken, the actual microbiota balance in the gut of the chicken, as well as the sex and age of chickens **(Aliakbarpour et al., 2012)**.

In the present study, body weight was increased in probiotic-supplemented group compared with that in control and propolis-supplemented group (Table 3), but no significant difference was detected ($p \geq 0.05$).

Many studies have confirmed the positive effect of probiotics on meat performance of broiler chickens. In the study of **Apata (2008)**, addition of probiotic preparation based on *Lactobacillus bulgaricus* to the basal diet (20, 40, 60 and 80 $mg.kg^{-1}$) resulted in improved performance of broiler chickens (35 day-old). Among the dietary treatments, 60 $mg.kg^{-1}$ probiotic preparation elicited the best performance of broiler chickens.

Similar results were observed in the previous study of **Zulkifli et al., (2000)**, who reported that dietary supplementation with *Lactobacillus* cultures improves the performance of chickens.

The significant increase ($p \leq 0.05$) in body weight was demonstrated also by **Ahmed et al., (2014)**, who investigated the effects of *Bacillus amyloliquefaciens* probiotic on growth performance of broiler chickens fed for 35 days. Increasing concentration of probiotic had positive linear effect on the body weight of broilers, with the highest values being observed in broilers offered 20 $g.kg^{-1}$ probiotic.

On the contrary, **Ghasemi et al., (2014)** observed the significant increase ($p \leq 0.05$) in body weight of male broilers only after synbiotic supplementation (probiotic in combination with prebiotic). In the study, the basal diet supplemented with 1 $g.kg^{-1}$ probiotic (combination of *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Enterococcus faecium*) did not result in significant effects on body weight of chickens compared to the control. The findings indicate that after probiotics + prebiotics supplementation may be achieved much better effects on performance of broilers.

Table 3 Effect of propolis extract and probiotic on performance and carcass characteristics of broiler chickens.

Parameter	Group	x	*SD*	SEM	CV (%)
Live body weight (g)	C	2270.20	107.88	34.11	4.75
	E1	2316.90	106.12	33.56	4.58
	E2	2335.00	107.37	33.96	4.60
Carcass weight (g)	C	1629.80	73.64	23.29	4.56
	E1	1669.10	102.48	32.41	6.14
	E2	1674.00	99.54	31.48	5.95
Giblets weight (g)	C	152.08	19.83	6.27	13.04
	E1	155.64	11.53	3.45	7.41
	E2	161.21	12.26	3.88	7.61
Carcass yield (%)	C	78.54	1.41	0.45	1.80
	E1	78.31	1.18	0.37	1.50
	E2	78.58	1.50	0.47	1.91
Abdominal fat (g)	C	22.14^{a}	4.77	1.51	21.54
	E1	21.85^{b}	6.48	2.05	26.66
	E2	24.70^{ab}	7.59	2.40	30.74
Liver (g)	C	40.91	4.63	1.46	11.31
	E1	40.61	5.46	1.73	13.44
	E2	44.50	7.09	2.24	15.93
Gizzard (g)	C	26.00	5.62	1.78	21.62
	E1	25.09	3.30	1.04	13.15
	E2	25.40	4.82	1.52	18.96
Heart (g)	C	10.72	1.10	0.35	10.25
	E1	10.88	1.49	0.47	13.67
	E2	10.77	1.73	0.55	16.10

Legend: C – control group; E1, E2 – experimental groups; x – arithmetic mean; *SD* – standard deviation; SEM – standard error of mean; CV – coefficient of variation; a, b – means with different superscripts within a column differ significantly ($p \leq 0.05$).

The positive effect of probiotic supplementation ($p \leq 0.05$) was reported in the study of **Aliakbarpour et al., (2012)**. The researches demonstrated that supplementation of either *Bacillus subtilis* as the mono-strain probiotic or *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* as the multi-strain probiotic in the feed mixture has the same potent stimulatory effects on broiler performance. Mono-strain probiotic fed broilers (2672.23 g), as well as multi-strain probiotic fed broilers (2664.92 g), had after 42 days of fattening higher body weight compared with control chickens (2608.99 g).

In the study of **Naseem et al., (2012)**, probiotic supplementation in two different doses (50 and 150 g per 1 ton of feed mixture) resulted in higher ($p \leq 0.05$) and similar body weight of broiler chickens (2141 g and 2120.3 g, respectively) compared with control chickens fed a basal diet (1962.1 g). The probiotic preparation consisted of *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus salivarius*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopessii*.

In another study, **Khaksefidi and Rahimi (2005)** also found significant increase ($p \leq 0.05$) in live body weight of chickens. On the one hand, the body weight of chickens in the experimental group (1700 g) at the end of fattening (42 days) was higher than that in the control (1620 g), but on the other hand it was markedly lower than that in the present study. The probiotic preparation used in the study of **Khaksefidi and Rahimi (2005)** consisted of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Aspergillus oryzae*, *Streptococcus faecium* and *Torulopsis* spp. and was fed at 100 $mg.kg^{-1}$ diet. The different results may be thus caused by the dosage and strain of probiotics.

Alkhalf et al., (2010) reported that administration of probiotic (*Pediococcus acidilactici*) in chickens appeared to have noticable effect ($p \leq 0.05$) on final body weight of broiler chickens, which was as low as that in the study of **Khaksefidi and Rahimi (2005)**. Chickens fed on probiotic levels 1 and 0.8 $g.kg^{-1}$ diet (1863.6 and 1844 g, respectively) exhibited higher body weight than control chickens (1661.31 g).

The beneficial effect of probiotic supplementation on chicken diet in terms of increased body weight (2372.50 vs. 1997.5 g) was also observed in the study of **Kabir et al., (2004)**. The probiotic preparation consisted of *Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus, Enterococcus aecium, Aspergillus oryzae* and *Candida pintolopessi*. It is important to note that broilers were administered the probiotic by drinking water application (consistent with present study).

Promising effect of probiotic ($p \leq 0.05$) as alternative for antibiotics was demonstrated by **Ghahri et al., (2013)**. They used the same probiotic preparation that was used in the study of **Kabir et al., (2004)**. The probiotic (applied into feed mixture) in two different doses increased live body weight of chickens (2475.13 and 2491 g) compared with that of the control group (2243.09 g). The most significant effect ($p \leq 0.05$) was, however, observed in synbiotic-supplemented group compared with that of other groups, which is in agreement with the results of **Ghasemi et al., (2014)**.

Contrary to the above-mentioned studies, no significant effect was observed in the study of **Brzóska et al., (2012)** and **Swiatkiewicz et al., (2014)**, whereas **Ritzi et al., (2014)** found even the negative effect of probiotic supplementation (*Bifidobacterium animalis* subs. *animalis*, *Lactobacillus salivarius* subs. *salivarius* and *Enterococcus faecium*) on performance of broiler chickens.

Regarding carcass yield, neither supplementation of the diet with propolis extract (78.31%), nor the probiotic preparation (78.31%) had any effect on carcass yield of broiler chickens compared to the control (78.54%). Yet, carcass yield of chickens in the present study was higher in comparison to other studies.

Our carcass yield results are consistent with those of **Tatlı Seven et al., (2008)** (76 – 77% vs. 75%), slightly lower were observed in study of **Attia et al., (2014)** (72.1% vs. 68.9%).

Also, **Swiatkiewicz et al., (2014)** reported similar carcass yield, which was, however, not affected when chickens were fed a probiotic bacteria (*Lactobacillus salivarius*) (74.89 vs. 75.53%).

Daneshmand et al., (2015) found much lower carcass yield, 62.77% in the probiotic-supplemented group, 62.86% in the propolis-supplemented group, and 62.93% in probiotic + propolis-supplemented group, that was, however, still higher than that in control (61.9%).

The effects of propolis extract and probiotic supplementation on composition, cholesterol content and energy value of meat of Ross 308 broiler chickens are shown in Table 4. It is evident that the parameters were not absolutely affected by dietary propolis extract and probiotic supplementation.

The results for meat samples of chickens fed the diet with propolis extract and probiotic were similar to those fed the basal diet, which is consistent with results of some experiments where various supplements were used. However, the significant changes ($p \leq 0.05$) were observed in some parameters.

As has been shown by our study, propolis supplementation was the most favourable among the groups, namely as for fat content in both breast (0.93 $g.100\ g^{-1}$) and thigh (9.62 $g.100\ g^{-1}$) muscles, the ash content in both breast (1.19 $g.100\ g^{-1}$) and thigh (1.05 $g.100\ g^{-1}$) muscles, the cholesterol content in breast muscle (86.42 $mg.100\ g^{-1}$), and the energy value in both breast (408.99 $kJ.100\ g^{-1}$) and thigh (664.8 $kJ.100\ g^{-1}$) muscles. Besides, the propolis-supplemented group showed low crude protein content in both breast (22.33 $g.100\ g^{-1}$) and thigh (18.05 $g.100\ g^{-1}$) muscles when compared with the other groups. As regards the probiotic-supplemented group, there was negative effect on the fat content (1.11 $g.100\ g^{-1}$), as well as the cholesterol content (92.17 $mg.100\ g^{-1}$), and the energy value (415.62 $kJ.100\ g^{-1}$) in breast muscle observed. It is noteworthy that the cholesterol content depends mainly on the type of muscle not the diet.

Regarding the meat composition of broiler chickens, some researchers have observed significant positive effects of natural feed supplements, whereas others reported no effect on the meat composition.

Table 4 Effect of propolis extract and probiotic on proximate composition, cholesterol content and energy value of chicken meat.

Parameter	Group	x	*SD*	SEM	CV (%)
Breast muscle					
Dry matter (g.100 g^{-1})	C	25.11	0.24	0.07	0.95
	E1	24.94	0.39	0.11	1.55
	E2	25.05	0.38	0.11	1.50
Crude protein (g.100 g^{-1})	C	22.52	0.40	0.11	1.76
	E1	22.33	0.58	0.17	2.61
	E2	22.32	0.28	0.08	1.25
Fat (g.100 g^{-1})	C	1.01^{ab}	1.13	1.04	13.02
	E1	0.93^{a}	0.10	0.03	11.28
	E2	1.11^{b}	0.12	0.03	10.66
Ash (g.100 g^{-1})	C	1.18^{ab}	0.03	8.7.10^{-3}	2.56
	E1	1.19^{a}	9.85.10^{-3}	2.84.10^{-3}	0.83
	E2	1.17^{b}	0.01	4.14.10^{-3}	1.22
Cholesterol (mg.100 g^{-1})	C	87.06	8.86	3.62	10.18
	E1	86.42	4.37	1.78	5.05
	E2	92.17	4.59	1.87	4.98
Energy value (kJ.100 g^{-1})	C	415.46^{a}	6.10	1.76	1.47
	E1	408.99^{b}	7.17	2.07	1.75
	E2	415.62^{a}	6.85	1.98	1.65
Thigh muscle					
Dry matter (g.100 g^{-1})	C	29.50	1.37	0.40	4.65
	E1	29.22	0.40	0.11	1.37
	E2	29.10	0.60	0.17	2.05
Crude protein (g.100 g^{-1})	C	18.48^{a}	0.21	0.06	1.17
	E1	18.05^{b}	0.34	0.10	1.88
	E2	18.06^{b}	0.21	0.06	1.16
Fat (g.100 g^{-1})	C	9.81	1.43	0.41	14.54
	E1	9.62	0.40	0.11	4.16
	E2	9.80	0.78	0.22	7.92
Ash (g.100 g^{-1})	C	1.02^{a}	0.02	6.38.10^{-3}	2.16
	E1	1.05^{b}	9.84.10^{-3}	2.84.10^{-3}	0.94
	E2	1.02^{a}	0.02	6.66.10^{-3}	2.27
Cholesterol (mg.100 g^{-1})	C	121.25	7.50	3.06	6.19
	E1	118.68	7.68	3.14	6.47
	E2	113.08	10.70	4.37	9.47
Energy value (kJ.100 g^{-1})	C	679.44	54.45	15.72	8.01
	E1	664.80	13.43	3.88	2.02
	E2	671.89	28.34	8.18	4.22

Legend: C – control group; E1, E2 – experimental groups; x – arithmetic mean; *SD* – standard deviation; SEM – standard error of mean; CV – coefficient of variation; a, b – means with different superscripts within a column differ significa ($p \leq 0.05$).

In the study of **Hossain et al., (2014)**, addition of 0.5% fermented water plantain (*Alisma canaliculatum*) increased the crude protein content in both breast and thigh muscles (24.99 and 23.19%, respectively) compared with the control (24.42 and 21.65%, respectively).

The results coincide with the findings of **Skřivan et al., (2012)**, who reported the highest protein content and the lowest fat content in the thigh muscle of broilers fed a diet with vitamin C (720 and 218 g.kg^{-1} of dry mater, respectively) and broilers fed a diet with selenite (724 and 216 g.kg^{-1} of dry mater, respectively). The results are similar to those in the present study (when converting into g/100 g).

Ahmed et al., (2015) found significantly higher crude protein content ($p \leq 0.05$) in the group of broilers fed a diet supplemented with pomegranate in breast (28.55%), as well as thigh muscle (23.44%) than that in non-supplemented group (26.21 and 22.18%, respectively). Moreover, there was a significant decrease ($p \leq 0.05$) in cholesterol content of breast muscle in the pomegranate-supplemented group (62.8 mg.100 g^{-1}) compared with the control (77.44 mg.100 g^{-1}).

On the contrary, **Swiatkiewicz et al., (2014)** noted no effect on the composition of breast muscle after probiotic supplementation, whereby the probiotic-supplemented group has shown the values very similar to the other groups, with a crude protein content of 23.5%.

Also, the probiotic supplementation in the study of **Haščík et al., (2011)** did not influence the composition of chicken meat significantly despite the slight positive effect in the probiotic-supplemented groups when compared with the control. The researchers have obtained the results similar to those in the present study.

To sum up the previous studies concerning the composition of chicken meat, there is a positive effect on fat content after natural feed additives observed in most of them, while the effect on protein content is not so noticeable.

CONCLUSION

The results of our study demonstrated that none of the experimental supplements (propolis extract and probiotic preparation based on *Lactobacillus fermentum*) caused a significant changes ($p \geq 0.05$) in performance and carcass characteristics of Ross 308 broiler chickens. However, the data have shown positive effect of propolis extract and probiotic due to the higher values of all the investigated parameters (especially in probiotic-supplemented group) than those in the control. The positive fact highlights the importance of evaluating the administration level of supplements in order to maximize the efficacy. As far as proximate composition, cholesterol content and energy value are concerned, there was a significant change ($p \leq 0.05$) in fat, ash and cholesterol content, as well as energy value in both breast and thigh muscles after the propolis supplementation. On the contrary, the probiotic supplementation was rather adverse for meat composition. Therefore, we assume that probiotic supplementation is more applicable for the performance and carcass characteristics, whereas the propolis supplementation is more applicable for meat composition of Ross 308 broiler chickens. Overall, further studies are needed to investigate the effect of the supplements.

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