JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

THE EFFECT OF BEE PRODUCTS AND PROBIOTIC ON MEAT PERFORMANCE OF BROILER CHICKENS

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doi: 10.15414/jmbfs.2019.9.1.88-92

ARTICLE INFO

Received 17. 1. 2019
Revised 19. 3. 2019
Accepted 19. 3. 2019
Published 1. 8. 2019

Regular article

OPEN ACCESS

ABSTRACT

The aim was the evaluation of meat performance in chickens Ross 308 after the addition of bee pollen and propolis in a combination with probiotic into diet for broiler chicken. A total of 180 Ross 308 broiler chickens of male and female sex were randomly divided into 3 groups (n=60): the control group (C) without additional supplementation, experimental group E1 supplemented with 400 mg bee pollen extract per 1 kg of feed mixture and 3.3 g probiotic (*Lactobacillus fermentum*) added to drinking water on a daily basis, and experimental group E2 supplemented with 400 mg propolis extract/1 kg feed mixture and 3.3 g probiotic (*L. fermentum*) added to drinking water on a daily basis. The feed mixtures were produced without any antibiotics and coccidiostatics. The fattening period lasted for 42 days. The findings of the work on the meat performance and carcass characteristics of chickens revealed that bee pollen in combination with probiotic was the most suitable feed supplement. Among the most noteworthy parameters affected positively ($P \leq 0.05$) by this supplement in comparison with control may be mentioned the breast part weight. Moreover, it seems likely that there was synergistic effect of bee pollen and probiotic manifested by higher live body and carcass weight ($P \leq 0.05$) in comparison with the control. Present results would also seem to suggest that propolis supplementation with probiotic had effects on meat performance and carcass characteristics of Ross 308 broiler chicken.

Keywords: chicken meat, bee pollen, propolis, probiotic, meat performance

INTRODUCTION

Chickens are the most popular amongst different poultry species worldwide and globally, there is increasing demand for poultry products. This may be attributed to the alleged healthiness of chicken, higher profit margins over a short period, and high acceptability of poultry products in many culinary traditions **(Rama Rao *et al.*, 2006; Sola-Ojo *et al.*, 2013; Haščík *et al.*, 2016)**. Poultry meat is an important source of nutrients for consumers worldwide. Generally speaking, consumers have become to focused on healthy food with a good taste and relevance to nutritional physiology. Furthermore, they have become concerned of potentially harmful additives such as drug residues, allergenic components, intoxicants, and microbial contamination, which may contribute to global health issues **(Grashorn, 2007)**. Nutritional composition of poultry meat is a result of birds' diet, particularly in the early stages of their life. Knowing the nutritional composition plays a key role in deciding whether the poultry meat can be part of a healthy dietary pattern **(Probst, 2009)**.
Sub-therapeutic doses of antibiotics are widely used in animal feeds as growth promoters to increase performance and animal production, to improve animal health, as well as to effectively regulate pathogens. Nevertheless, due to possible adverse effects of antibiotics, such as bacterial resistance and the presence of antibiotic residues in the final product, the European Commission in 2006 banned the use of antibiotics as growth promoters **(Attia *et al.*, 2014; Abou-Zeid *et al.*, 2015)**. As such, new feed additives of plant origin regarded as natural products acceptable by consumers have been proposed by animal breeders **(Kahraman *et al.*, 2016)**. In this regard, many researchers have attempted to carry out experiments to study natural feed additives for broilers such as herbs, spices, various plant extracts, antioxidants, enzymes, probiotics and prebiotics as possible antibiotic growth promoter substitutes that could be used in poultry diet in order to reduce possible harmful effects of antibiotics **(Khaksefidi and Rahimi, 2005; Khattak *et al.*, 2006; Toghyani *et al.*, 2011; Abdel-Kareem and El-Sheikh, 2015; Abo Omar *et al.*, 2016; Farag and El-Rayes, 2016; Karadas *et al.*, 2016; Ricke, 2018)**. Recently, propolis and bee pollen have been also considered as possible new feed additives **(Kleczek *et al.*, 2012; Attia *et al.*, 2014; Abou-Zeid *et al.*, 2015; Klarić *et al.*, 2018)**.
Bee products have been extensively used in traditional folk medicine. There is a new promising insight in the research for bee products , such as propolis, pollen, bee venom, honey, and royal jelly **(Seven *et al.*, 2014).**
Bee pollen is defined as an agglomerate of pollen from diverse flowers and collected from plant anthers by honey bees (*Apis mellifera* L.). Bee pollen contains a variety of biomolecules such as essential amino acids, proteins, unsaturated fatty acids, anthocyanins, organic acids (ferulic, pantothenic, etc.), vitamins, minerals (iron, manganese, zinc) and trace elements **(Oliveira *et al.*, 2013; Attia *et al.*, 2014)**. It also contsists of carotenoids, flavonoids and phytosterols **(Feás *et al.*, 2012)**. Bee pollen is regarded as a health promoting food additives with a wide range of therapeutic properties, among which antioxidant, antifungal, antimicrobial, anti-radiation, chemoprotective, chemopreventive, hepatoprotective, and anti-inflammatory activity are the most prominent **(Yamaguchi *et al.*, 2006; Pascoal *et al*., 2014).** Bee pollen has been reported to promote animal growth, to improve the quality and security of animal products, to enhance the immunizing function of poultry and to protect the intestinal tract **(Liu *et al.*, 2010).** The exact composition of bee pollen significantly depends on the plant source as well as other factors such as climate, beekeeper activities and soil type **(Morais *et al*., 2011).**
Propolis is a sticky gummy resinous material that worker honeybees (*Apis mellifera* L.) collect from buds and young shoots of certain trees and shrubs and mix it with wax and salivary enzymes **(Greenaway *et al.*, 1990; Aygun *et al.*, 2012).** Propolis contains about 300 constituents **(Seven *et al.*, 2012)** and its chemical properties and composition depend on the various types of pollens, vegetation, from which the honey bee insects have collected the exudates. A detailed analysis of pollen has revealed a wide array of biomolecules including polyphenols (flavonoid aglycones, phenolic acids, aldehydes, ketones, and alcohols), steroids, terpenoids, amino acids and inorganic compounds **(Dimov *et al.*, 1991; Marcucci *et al.*, 2000; Moreno *et al.*, 2000; Trusheva *et al.*, 2006; Gardana *et al.*, 2007; Nolkemper *et al.*, 2010)**. Moreover, propolis is composed

of minerals (Mg, K, Ca, Cu, Na, Mn, Zn and Fe), vitamins (B_1, B_2, B_6, C and E), as well as fatty acids and enzymes **(Lotfy, 2006).** All these bioactive components of propolis contribute to its antibacterial, antiviral, antifungal, antiprotozoal, antimicrobial, analgesic, anti-inflammatory, antioxidant, anaesthetic, cytostatic, immunostimulant and immunomodulatory effects in both human and animal health **(Talas and Gulhan, 2009**; **Seven *et al*., 2012; Aygun *et al*., 2012; Eyng *et al*., 2013; Fan *et al*., 2013).** Propolis administration has been used in poultry diet **(Seven, 2008)** and according to some reports, propolis may be an effective natural alternative to antibiotic growth promoters in poultry nutrition **(Biavatti *et al*., 2003, Denli *et al*., 2005; Shalmany and Shivazad, 2006; Seven *et al*., 2008; Mathivanan *et al*., 2013; Babaei *et al*., 2016).**

An alternative approach to the administration of subtherapeutic antibiotics in chicken diet represents the use of probiotics **(Alkhalf *et al*., 2010).** Probiotics are live, non-pathogenic bacteria that have the ability to contribute to normal health and milieu of the intestinal tract **(Giannenas *et al*., 2012).** Among the most compelling advantages of probiotics are no residues in animal production and no antibiotic resistance by consumption **(Alkhalf *et al*., 2010).** The major probiotic strains include *Saccharomyces*, *Lactobacillus*, *Bacillus*, *Streptococcus* and *Aspergillus spp*. **(Tannock, 2001)**. The selected microbial strain, preparation method, dosage, condition of animals and the number of viable microorganisms in probiotics are considered to be critical factors affecting the efficiency of probiotics **(Panda *et al*., 2005)**.

Several studies have emphasized that probiotics in broiler diets have the ability to improve the nutrition, health and growth performance when compared with non-supplemented diets, being possibly as effective as antibiotic growth promoters **(Wallace and Chesson, 1995**; **Gong *et al*., 2002; Kalavathy *et al*., 2003**, **Mountzouris *et al*., 2010, Shim *et al*., 2010).**

The focus of the present study was to evaluate the meat performance following the addition of bee pollen and propolis in a combination with probiotic into diet for Ross 308 broiler chicken.

MATERIAL AND METHODS

Animals and experimental design

The experiment was realized in the test poultry station of the Slovak University of Agriculture (SUA) in Nitra. The fattening period lasted for 42 days and the animals were kept under the same conditions. The experiment included 180 one-day-old chicks (Ross 308) of mixed sex randomly divided into 3 groups (each containing 60 chickens). The size of pen for one group of chickens was 3.2 x 2.4 m. 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 23 °C thereafter. The temperature and relative humidity were controlled. Over the entire fattening period, the chickens were provided with *ad libitum* access to feed (mash form) as well as drinking water and were kept under a constant light regime. Probiotic dosing pattern via drinking water is presented in **Table 1**.

Table 1 Probiotic dosing pattern via drinking water

Week of age	Amount of water per day for 60 chickens (L)	Amount of probiotic pre day for 60 chickens (g)
1.	2.49	3.3
2.	3.51	3.3
3.	4.59	3.3
4.	6.69	3.3
5.	8.61	3.3
6.	10.59	3.3

Diets were prepared to accommodate the nutrient requirements of broilers following the recommended reference levels **(Bulletin of MARD SR, 2005)**, and broilers were subjected to a two phase feeding programme, starter HYD-01 (1 – 21 d) and grower HYD-2 (22 – 42 d) diets. The composition of basal diets is presented in **Table 2**. The starter and grower feed mixtures were produced without any antibiotics and coccidiostatics and were prepared by Biofeed, Inc. (Kolárovo, Slovak Republic). The experimental groups were set up as follows: the control group (C) involved the basal diet without supplementation; the experimental group of chickens (E1) was fed with basal diet plus 400 mg bee pollen extract/1 kg of feed mixture and 3.3 g probiotic (*Lactobacillus fermentum*) added daily to the drinking water and chicks in experimental group E2 were fed with a complete feed mixture plus 400 mg propolis extract/1 kg of feed mixture and 3.3 g probiotic (*Lactobacillus fermentum*) added daily to the drinking water.

Table 2 Composition of feed mixtures

Ingredients (%)	Starter (HYD-01) (1^{st} – 21^{st} day of age)	Grower (HYD-02) (22^{nd} – 42^{th} day of age)
Maize	36.00	41.00
Wheat	34.00	34.00
Soybean meal (48% N)	21.20	18.60
Fish meal (71% N)	3.90	2.10
Dried blood	1.20	1.20
Ground limestone	1.05	1.10
Monocalcium phosphate	1.00	0.75
Sodium bicarbonate	0.14	0.20
Fodder salt	0.11	0.10
Methionine	0.15	0.23
Lysine	0.05	0.06
Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 0.5%*	0.50	0.50
Nutrient composition [g.kg^{-1}]		
Linoleic acid	13.52	14.14
Fibre	30.29	29.83
Crude protein	211.38	190.56
Ash	25.81	20.32
Ca	8.17	7.25
P	6.79	5.75
Mg	1.43	1.41
ME_N (MJ.kg^{-1})	12.03	12.04

*active substances per kilogram of premix: vitamin A 2,500,000 IU; vitamin D_3 800,000 IU; vitamin E 50,000 mg; ascorbic acid 50,000 mg; niacin 12,000 mg; D-pantothenic acid 3,000 mg; riboflavin 1,800 mg; pyridoxine 1,200 mg; methadione 800 mg; thiamine 600 mg; folic acid 400 mg; choline 100,000 mg; biotin 40 mg; cobalamin 10.0 mg; betaine 50,000 mg; Mn 20,000 mg; Zn 16,000 mg; Fe 14,000 mg; I 200 mg; Cu 2,400 mg; Co 80 mg; Se 50 mg. ME_N – metabolizable energy corrected for nitrogen equilibrium.

Characterization of probiotic preparation applied in experiment

In the experiment, two-component probiotic preparation "Propoul" containing probiotic microorganism *Lactobacillus fermentum* (1.10^9 CFU per 1 g of bearing medium) and a potentiating component (maltodextrin and oligofructose) was used. The probiotic preparation was supplied by IPC Ltd. (Košice, Slovak Republic).

Bee pollen and propolis extracts preparation

Bee pollen and propolis extracts used in the experiment came from the Slovak Republic. The extracts were prepared from minced bee pollen and propolis in 80% ethanol inside of 500 ml flasks, according to **Krell (1996).** The extraction took place in a water bath at 80 °C under reflux cooler for one hour. Subsequently the extracts were cooled and centrifuged. The obtained supernatants were evaporated in a rotary vacuum evaporator at 40 - 50 °C, and then weighed. Finally, the residues in an appropriate amount (depending on addition of supplement per kg of feed) was dissolved in ethanol and applied to the feed mixture through a bearing medium.

Slaughter and measurements

At 42 days of age, 30 chickens of mixed sex (15 ♂ and 15 ♀) were selected from each group based on the average weight, then weighed and slaughtered at the experimental slaughterhouse of Department of Animal Products Evaluation and Processing (SUA, Nitra). The chickens were slaughtered by conventional neck cut, bled, feathers removed, and eviscerated.

Examined parameters in experiment were as follows: live body weight (BW) (g) at the and fattening period; carcass weight (CW) (g); giblets weight (g); beast part weight (g); thigh part weight (g); abdominal fat weight (g); total weight of internal fats (heart, gastric, and abdominal) (g); carcass yield (%); breast part yield (% of CW); thigh part yield (% of CW); valuable carcass parts (breast and thigh) yield (% of CW).

Statistical analysis

Statistical analysis was calculated using ANOVA and SAS software with the Enterprise Guide 4.2 application (version 9.3, SAS Institute Inc., USA, 2008). Results were reported as mean±standard deviation. Statistical significance was calculated using the t-test. Differences between the groups were considered significant at P≤0.05.

RESULTS AND DISCUSSION

The use of growth promoters as antibiotics for poultry production has been banned in many countries, which in turn comes hand in hand with their prohibition as possible protective agents against infectious diseases and subsequently an increased economic loss for the poultry industry (**Peric *et al.*, 2009).** Therefore, many researchers have tried to search for natural feed additives such as bee products as propolis, bee pollen and probiotics to be used in the poultry diet in order to reduce possible harmful effects **(Hegazi *et al.*, 2012).**
The effect of bee supplements in feed as bee pollen and propolis in combination with probiotic on meat performance of Ross 308 broiler chickens is shown in **Table 3**.

Table 3 Effect of natural feed supplements on meat performance and carcass characteristics of broiler chickens

Parameter\Group	C	E1	E2
Live BW (g)	2,270.2±107.88^{a}	2,401.7±144.5^{b}	2,358±157.16^{ab}
CW (g)	1,629.8±73.65^{a}	1,714±82.74^{b}	1,688±121.07^{ab}
Giblets (g)	152.08±19.83	162.18±17.31	159.12±16.33
Breast part (g)	621.34±52.22^{a}	667.48±43.21^{b}	641.94±53.18^{ab}
Thigh part (g)	471.74±45.11	474.38±47.79	481.39±47.04
Abdominal fat (g)	22.14±4.77	25.01±5.63	24.81±6.81
Total internal fats (g)	31.64±4.94	34.52±6.55	32.79±8.94
Carcass yield (%)	78.54±1.41	78.17±1.65	78.33±0.86
Breast part yield (% of CW)	38.07±2.23	38.98±2.41	38.01±1.37
Thigh part yield (% of CW)	28.93±2.42	27.64±1.88	28.5±1.51
Valuable parts yield (% of CW)	67±2.11^{ab}	66.61±2.02^{b}	66.51±1.82^{b}

Note: Values are given as mean ± SD (standard deviation); n = 30; C = control group; E1, E2 = experimental groups; $^{a, b}$ = means within the same row with different superscripts differs significantly (P≤0.05); BW = body weight; CW = carcass weight.

According to the data obtained, significant differences (P≤0.05) in live body weight and carcass weight were found between broilers fed a basal diet (C) and those having bee pollen plus probiotic in their diets (E1) (2,401.7 and 1,714 g vs. 2,270.2 and 1,629.8 g, respectively). Both live body weight and carcass weight in E1 group were the highest among the groups. The increase in carcass weight is in large part due to the increasing in body weight.
It is also apparent from this table that C group had significantly (P≤0.05) lower breast part weight (621.34 g) compared with group E1 (667.48 g). Contrary to expectations, it was a little disappointing that the analysis did not confirm any differences (P>0.05) between the groups with respect to the other carcass characteristics (giblets weight, thigh part weight, abdominal fat weight, total weight of internal fat, carcass yield, breast part yield, thigh part yield and valuable parts yield). Our results are also consistent with the findings of other authors **(Alloui *et al.*, 2012; Mokhtari *et al.*, 2015; Odefemi, 2016; Pourakbari *et al.*, 2016)** that revealed no significant effect (P>0.05) on the breast and thigh percentage of broilers fed diet containing probiotic.
On the other hand, bee pollen and probiotic supplementation has shown to be the most favourable among the dietary treatments regarding meat performance and carcass characteristics of broilers. Among the most noteworthy parameters affected positively by this supplement may be mentioned the live body weight, carcass weight and breast part weight. These improvements may be due to the nutritive value of bee pollen as a rich source of protein, EAAs, MUFA and PUFA, and the presence of minerals and the other micronutrients with a positive impact on the broiler health and metabolism. These results would also seem to suggest that propolis supplementation with probiotic (E2) had hardly any effect on performance and carcass characteristics of broiler chickens Ross 308. Despite this, it cannot be ignored that there was a tendency for propolis in combination with probiotic (E2) to increase (P>0.05) the parameters of meat performance compared with C group. It is difficult to explain this result, but it might be related to components in propolis such as benzoic and 4-hydroxybenzoic acid, which may improve the digestibility of such nutrients as protein and ash **(Seven *et al.*, 2012)**. Another possible explanation for this might be that propolis contains substances with antimicrobial potential, also resulting in a better intestinal digestion and absorption **(Shaddel-Tili *et al.*, 2017)**. Naturally, there may be other possible explanations. Disappointingly, the findings hardly show any synergic effect of propolis and probiotic (E2).

Hardly any studies have been published on combined effect of bee products and probiotics. **Adhikari *et al.* (2017)** found decreased live body weight (P>0.05) of broilers Cobb 500 fed with different amounts of native bee pollen plus probiotics (commercial preparation containing *Saccharomyces cerevisiae*, *S. boulardii*, *Lactobacillus acidophilus* and *Propionibacterium freudenreichii*). Another study **(Daneshmand *et al.*, 2015)** evaluated the effects of propolis (200 mg.kg^{-1} of feed) and probiotic (450 mg.kg^{-1} of feed) alone or their combination (200 and 450 mg.kg^{-1}, respectively) on performance of male broilers (Ross 308). A commercial product containing *L. acidophilus*, *L. casei*, *Bifidobacterium bifidum* and *Enterococcus faecium* was used as the source for the probiotic. However, none of the supplements affected (P>0.05) body weight at 42 d of age in comparison with the control group. The improved weight gain might be caused by the nutrient composition of bee pollen along with their antioxidant activities and health protection **(Szczêsna, 2007; Šarić *et al.*, 2009)** as well as by maintaining the intestinal microbial balance of gastrointestinal tract, the activity of digestive enzymes which may help to enhance the production performance **(Tortuero and Fernandez, 1995; Jin *et al.*, 1997).** The study of **Klarić (2014)** demonstrated improved performance (P≤0.05) of broilers after both bee pollen and propolis supplementation (separately or in combination). Regarding the probiotics, there is a considerable variation in the available studies focused on the effect of probiotic strains on the performance of broiler chickens. The inconsistent and contradictory reports are due to many factors that affect the response of broiler chickens to probiotics. The factors include method and duration of probiotic feeding, nature and dose of the administered strains and their persistence, variations in the physiological condition of the animal, the actual microbial balance in the chicken gut, overall diet, age and sex of chicken, as well as overall farm hygiene, and environmental stress factors **(Zhou *et al.*, 2010; Aliakbarpour *et al.*, 2012)**. Similarly, findings obtained in other reports showed that probiotics, either as a single strain or as a mixture of strains, promoted (P≤0.05) the performance and carcass characteristics of broilers **(Khaksefidi and Rahimi, 2005; Alkhalf *et al.*, 2010; Zhou *et al.*, 2010; Aliakbarpour *et al.*, 2012; Naseem *et al.*, 2012; Taklimi *et al.*, 2012; Ghahri *et al.*, 2013; Shokryazdan *et al.*, 2017)**.

CONCLUSION

This study was designed to determine the effect of dietary supplementation with bee pollen, propolis, and probiotic (*Lactobacillus fermentum*) on the meat performance and carcass characteristics of broiler chickens. The findings of the work on meat performance and carcass characteristics of chickens indicated that bee pollen in combination with probiotic was the most suitable feed supplement. Among the most noteworthy parameters affected positively (P≤0.05) by this supplement in comparison with control may be mentioned the breast part weight. Moreover, it seems likely that there was synergistic effect of bee pollen and probiotic manifested by higher live body weight and carcass weight (P≤0.05) in comparison with the control.
Present results would also seem to suggest that propolis supplementation with probiotic had effects on meat performance and carcass characteristics of Ross 308 broiler chicken.

Acknowledgments: This work was supported by KEGA no. 027SPU-4/2019

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