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Production Parameters, microbiological Composition of Intestines and Slaughter Performance of Broilers fed with Bee Pollen

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Summary

The focus of the research was to examine the effects of the addition of different concentrations of bee pollen to broiler feed on production performance, microbiological composition of caecum and carcass quality. The study was performed on 1200 chickens divided into 5 groups with 6 replicates per group. During the test, the broilers were fed with two complete feed mixtures (starter and finisher) each differed only in the amount of pollen added: no pollen in control group (C); 0.25% pollen in feed consumed by P0.25 group; 0.5% in P0.5 group; 0.75% for group P0.75; and 1.0% of pollen in feed for P1.0 group. Body weight of the chickens was measured during the change of the mixture, on the 21st day and at the end of the research (day 42). Average feed consumption, average daily gain, feed conversion rate, mortality and European Production Efficiency Factor (EPEF) were determined. At the end of the experiment, 12 chickens of both sexes were sacrificed from each group, in order to determine the microbiological composition of the intestines and slaughter performance.

The results showed positive effects of pollen use on production parameters. Chickens of P0.75 group had significantly higher ($p < 0.05$) average daily gains and better feed conversion, also the value of EPEF was significantly higher ($p < 0.01$) in P0.75 group compared to groups with lower pollen addition. A significantly higher ($p < 0.01$) number of *Enterobacteriaceae* was found in the cecum content of chickens of C and P0.25 groups compared to P0.75 and P1.0 groups. No significant differences in slaughter performance were found. It was concluded that the addition of 0.75% pollen can have a positive effect on the production performance and the microbiological composition of the intestine.

Keywords: broiler chickens, diet, bee pollen

Zusammenfassung

Leistungsmerkmale, mikrobiologische Zusammensetzung des Darms und Schlachtleistungen von Broilern, die mit Bienepollen gefüttert worden sind

In dieser Untersuchung sollte die Wirkung einer Zugabe von Bienenpollen in unterschiedlicher Konzentration zum Futter auf die Produktionsleistung, die mikrobiologische Zusammensetzung des Blinddarms und die Schlachtkörperqualität von Broilern geprüft

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werden. Die Studie wurde an 1.200 Broilern durchgeführt, unterteilt in 5 Gruppen, mit 6 Wiederholungen je Gruppe. Während des Tests wurden die Broiler mit zwei komplett Futtermischungen (Starter und Finisher) gefüttert, die sich jeweils nur in der dem Futter zugesetzten Pollenmenge unterschieden: Kontrollgruppe (C): kein Pollen; P0.25-Gruppe: 0,25% Pollen; P0.5-Gruppe: 0,5%; P0.75-Gruppe: 0,75%; P1.0-Gruppe: 1,0% Pollen im Futter. Das Körpergewicht der Broiler wurde am 21. Tag (Versuchsbeginn) und am 42. Tag (Ende der Untersuchung) ermittelt. Es wurden der durchschnittliche Futterverbrauch, die durchschnittliche, tägliche Gewichtszunahme, die Futterverwertung, die Mortalität und der European Production Efficiency Factor (EPEF) bestimmt. Am Versuchsende wurden aus jeder Gruppe 12 Broiler beiderlei Geschlechts geschlachtet, um die mikrobiologische Zusammensetzung des Darms und die Schlachtleistung zu bestimmen.

Die Ergebnisse zeigten positive Auswirkungen des Polleneinsatzes auf die Produktionsparameter. Broiler der P0.75-Gruppe hatten signifikant höhere ($p < 0,05$) durchschnittliche Tageszunahmen und bessere Futterverwertung, auch der EPEF-Wert war in der P0.75-Gruppe signifikant höher ($p < 0,01$) im Vergleich zu den Tieren in den Gruppen mit geringerer Pollensubstitution. Eine signifikant höhere ($p < 0.01$) Anzahl von *Enterobacteriaceae* wurde im Blinddarmgehalt von Broilern der C und P0.25-Gruppe im Vergleich zu den P0.75- und P1.0-Gruppen festgestellt. Es wurden keine signifikanten Unterschiede in der Schlachtleistung festgestellt. Es ergab sich die Schlussfolgerung, dass sich die Zugabe von 0,75% Pollen positiv auf die Mast- und Schlachtleistungen sowie die mikrobiologische Zusammensetzung des Darms auswirken kann.

Schlüsselwörter: Broiler, Ernährung, Bienenpollen

1 Introduction

The ban on the use of antibiotics in poultry nutrition is in place due to the negative effects that are manifested through the occurence of bacterial resistance and their non-selective action in the digestive tract (destruction of beneficial bacteria). The consequence of not using antibiotics as a dietary supplement is the reduction of production performance, so the task of poultry industry is to find alternative solutions that will enable obtaining adequate quantities of a safe product, which is the request of consumers themselves.

Bee products, including pollen, are used in traditional medicine and have recently appeared as possible additives in poultry diets (Attia et al., 2014; Haščík et al., 2017). Pollen powder is the male gametes of flowering plants. These are small grains of light yellow to black color that honey bees collect and moisten with the secretion of salivary glands and form pollen balls. From the point of view of bees, pollen is the most important substance in the hive where it serves as a basic food for young bees. It contains everything that is necessary for life and good health and is a mix of proteins, fats, carbohydrates, amino acids and an abundance of vitamins, minerals and enzymes. The chemical composition of pollen depends on the type of plant from which the bees collect it. Due to the richness of protective substances, pollen can play a role in strengthening of the organism, preserving health and improving the immune system (Abdelnour et al., 2018). Studies in which pollen was used alone or in combination with other additives in a concentration of 0.5 to 2.5% (Fazayeli-Rad et al., 2015; Hosseini et al., 2016; Adhikari et al., 2017) confirm that the use of pollen in the diet of broiler chickens results in better production performance. Pollen can be beneficial for the cardiovascular system and reduce cholesterol and triglyceride levels (Klarić et al., 2018). It can also have antibacterial and anti-inflammatory effects and a positive effect on the level of bacteria in the intestines (Pascoal et al., 2014) as well as on the improvement of the condition of the digestive tract (Wang et al., 2007). There are studies in which the positive influence of pollen on the quality of chicken carcass has been determined (Abood and Ezzat, 2018). Demir and Kaya *(2020)* reports positive impact of the use of pollen in the diet for laying hens in a concentration of 0.5 to 1.5% in the mixture on production results and egg quality. Pollen was collected in the spring period. Sarić et al. (2009) state that pollen has a significant source of compounds with health protective potential and antioxidant activity. Having in mind all the mentioned beneficial effects of bee pollen, as well as the complex combination of bioactive components it contains, the hypothesis in the research was that the use of pollen powder in broiler chickens' diet can improve production performance, intestinal microbiological composition, health and carcass quality.

The focus of the study was to examine the effects of the addition of different concentrations of pollen procured from a local beekeeper to complete feed mixtures on production performance, microbiological composition of caecum and carcass quality of broiler chickens.

2 Materials and methods

2.1 Experimental design and nutrition

The study was performed on 1200 one-day-old Cobb 500 hybrid broiler chickens of both genders. The chickens were housed and divided into 5 treatments and 6 replicates per treatment, in 30 boxes of the same dimensions (2 m × 2 m, so that each bird had $0.1 m^2$ available). The gender ratio in each box was the same. During the research, broilers were fed with two complete feed mixtures (starter and finisher), based on corn/soybean, whose composition is shown in Table 1. Water and feed were *ad libitum*, and the mixture differed only in the amount of added pollen (on top of the basic composition). No pollen was added in control group (C). Chickens of the group (P0.25) consumed mixtures with the addition of 0.25% pollen, in group (P0.5) 0.5% was added, in group (P0.75) 0.75% and in group (P1.0) 1.0%. Pollen was procured from a local beekeeper (Sremska Mitrovica, Serbia). Pollen was obtained using special catchers placed at the entrance to the hive containing a collection container and a net that prevents bees from bringing pollen into the hive. The price of pollen at the time of purchase was 10 €/kg. Standard chemical analysis showed that the bee pollen used contained 92.1% dry matter, 19.7% crude protein, 4.2% crude fat, 3.5% crude fiber and 2.2% ash. Before being added to the chicken feed, the pollen was ground into a fine powder.

2.2 Production results

Recording of body weight of all chickens (individually) and feed consumption (per box) was performed during the change of mixture (day 21) and at the end of the study (day 42). Average broiler feed consumption, average broiler daily gain, mortality and feed conversion rate were determined for every box. The European Production Efficiency Factor (EPEF) was also determined based on the achieved average body weight, vitality, feed conversion ratio and fattening duration, whose calculation formula is: EPEF = (Liveweight, kg × Livability, % / Age of depletion, days × Feed Conversion Ratio, kg feed/kg gain) × 100.

2.3 Microbiological analysis of the contents of the cecum

At the end of the experiment, 1 male and 1 female (a total of 12 chickens per group) were selected from each box and slaughtered. Cecums were separated from the sacrificed

Table 1. Feed composition (g/kg) and calculated nutrient content (g/100 g as fed) of the basal diets used in trial
Futterzusammensetzung (g/kg) und berechneter Nährstoffgehalt (g/100 g wie verfüttert) der verwendeten Grundfutterarten

Ingredient, g/kg	Starter 0–21 d	Finisher 22–42 d
Corn	531	601
Full fat soybeans (extruded)	80	100
Vegetable oil	20	30
Soybean meal	330	230
Monocalcium phosphate	12	12
Limestone	14	14
Salt	2	2
Vitamin + mineral supplement[1]	10	10
DL-methionine	1	1
Total	1000	1000
Nutrients and energy level (calculated)		
ME, MJ/kg	12.7	13.3
Crude protein, %	21.9	18.9
Crude fat, %	5.92	7.45
Crude fibre, %	3.37	3.00
Lysine, %	1.24	1.02
Methionine + Cystine, %	0.72	0.55
Ca, %	1.00	0.95
P, %	0.65	0.62

[1]Contained per kg of diet: vitamin A, 1200 IJ; thiamine, 3.3 mg; riboflavin, 9.5 mg; niacin, 5.2 mg; pantothenic acid, 11 mg; pyridoxine, 4.2 mg; folic acid, 1.6 mg; vitamin B12, 0.02 mg; biotin, 0.02 mg; choline, 400 mg; vitamin D3, 5000 IJ; vitamin E, 55 mg; vitamin K3, 7 mg; Mn, 110 mg; Fe, 33 mg; Zn, 110 mg; Cu, 9 mg; I, 0.5 mg; Se, 0.2 mg.

chickens, which were placed in sterile bottles, and within 60 minutes, 1 g of the content was isolated from the samples in aseptic conditions, which was homogenized in physiological solution. After homogenization, preparation, seeding of selective nutrient media, incubation and reading were performed, as follows: testing of the total aerobic bacteria count was determined on agar (Plate count agar, Torlak) after incubation at 30°C for 72 h in aerobic medium; MacConkey agar No.3 at 37°C for 24 h in an aerobic environment was used to test for the presence and determine the total *Enterobacteriaceae* count; The total *Escherichia coli* count was determined on modified UTI agar (UTI agar, Himedia) after incubation at 37°C for 24 h in aerobic medium.

2.4 Slaughter characteristics

The selection of chickens for slaughter was done so that the individual body weight of the selected chickens from each box corresponded to the average body weight of that box, and the carcass quality traits were determined on the slaughtered chickens (1 male and 1 female from each box, 12 chickens per group, a total of 60 analyzed birds). After slaughter and manual processing, the chicken carcasses were cooled to 4°C for 24 hours, subsequently the carcass weight and the weight of abdominal fat were determined and the carcasses were cut into basic carcass parts and measured (breasts, drumsticks, thighs, wings). During the processing of the carcass, the weight of the liver, heart and gizzard was measured. The results of each measured carcass and parts are expressed relatively, as % of body weight of an individual broiler before slaughter.

2.5 Statistical data processing

The obtained data were processed using the software package STATISTICA (Stat Soft Inc, 2012). For production performance, pen (box) means was considered an experimental unit, and for statistical analysis of cecum and carcass characteristics, a slaughtered bird. Before statistical processing of data for microbiological analysis of the cecum, their transformation was performed using the logarithmic function log10 (x).

One-way ANOVA sofwere procedure was used for analysis of overal main effect of dietary treatment (with treatment as fixed effect), while the LSD test was used to determine the statistical significance of differences between individual mean values. Significance levels were set at $p < 0.05$.

3 Results and discussion

The effects of pollen addition in the diet of broiler chickens on production performance are shown in Table 2.

During the feeding of starter and finisher mixtures, it was determined that there were no significant differences in feed consumption, average daily gain and feed conversion ratio. At the level of the entire study period (days 1–42), it was established that with the increase of pollen content in mixtures to the level of 0.75%, the average daily gain increased and feed conversion ratio was better. Chickens of P0.75 group had significantly ($p < 0.05$) higher growth performance and better feed conversion ratio compared to groups with lower pollen participation. No significant differences in chicken mortality between groups were found during the study. As a result of more favourable feed conversion ratio and higher average daily gain, chickens of P0.75 group had significantly higher ($p < 0.01$) EPEF values compared to groups with lower pollen participation. With the increase of the share of bee pollen in the mixtures, the value of EPEF also increased to the level of participation of 0.75%.

The obtained results show the importance of the use of bee pollen, which is reflected in significantly higher daily gain and better food conversion ratio, as well as significantly higher EPEF values. In a study by Abood and Ezzat (2018) also statistically significantly higher body weight values are reported for chickens fed mixtures with 1% pollen in the mixture at the age of 35 days compared to the control. With the increase of the concentration of active pollen ingredients in our study to the level of participation of 0.75% in the mixture, a gradual increase of the daily gain and better food conversion ratio were obtained, and a gradual increase of EPEF was also established. Better growth of chickens fed mixtures with the addition of pollen occurred as a result of nutrients, primarily due

Table 2. Effects of different levels of addition of bee pollen to broiler diet on growth performance of chicks in two periods of fattening and production performance in the whole trial period
Auswirkungen unterschiedlicher Zugabemengen von Bienenpollen zum Broilerfutter auf die Wachstumsleistungen der Broiler in zwei Mastperioden und die Produktionsleistungen im gesamten Versuchszeitraum

	Treatments (Groups)						
	C	P0.25	P0.5	P0.75	P1.0	SEM	p
Starter period (1–21 d)							
FI, g/d	59.79 ± 1.22	59.38 ± 0.81	57.68 ± 1.95	60.65 ± 1.89	59.83 ± 2.09	0.337	0.060
ADG,g/d	40.19 ± 0.89	41.63 ± 1.15	41.18 ± 1.05	42.19 ± 1.93	41.72 ± 0.68	0.241	0.086
FCR, g/g	1.49 ± 0.05	1.43 ± 0.04	1.41 ± 0.03	1.45 ± 0.03	1.44 ± 0.07	0.009	0.072
Finisher period (22–42 d)							
FI, g/d	144.29 ± 3.12	144.60 ± 1.22	145.33 ± 2.89	141.61 ± 1.99	141.50 ± 5.49	0.630	0.163
ADG,g/d	74.01 ± 2.26	74.91 ± 3.64	75.81 ± 4.05	78.58 ± 2.87	76.98 ± 1.25	0.586	0.109
FCR, g/g	1.94 ± 0.0	1.94 ± 0.08	1.93 ± 0.10	1.81 ± 0.08	1.88 ± 0.34	0.034	0.062
Whole period (1–42 d)							
FI, g/d	102.25 ± 1.89	103.68 ± 0.74	102.37 ± 2.64	102.37 ± 1.17	103.45 ± 3.07	0.372	0.637
ADG, g/d	57.10^{b} ± 1.23	58.27^{b} ± 1.25	58.49^{b} ± 2.26	60.36^{a} ± 1.43	59.35^{ab} ± 0.85	0.323	**0.011**
FCR, g/g	1.78^{a} ± 0.06	1.76^{a} ± 0.03	1.75^{a} ± 0.05	1.68^{b} ± 0.06	1.72^{ab} ± 0.03	0.024	**0.022**
Mortality,%	2.08 ± 1.88	2.92 ± 1.02	2.50 ± 2.24	2.08 ± 1.88	2.92 ± 1.88	0.317	0.867
EPEF	320.01^{b} ± 10.93	328.07^{b} ± 11.47	333.28^{b} ± 19.28	358.31^{a} ± 23.96	341.01^{ab} ± 12.31	3.542	**0.004**

Values are given as mean ± SD (standard deviation); SEM, Standard error of the means; FI, feed intake; ADG, average daily gain; FCR, feed conversion rate; EPEF, European Production Efficiency Factor; ^{a,b} In a row, the least squares means with a different superscript differ significantly ($p < 0.05$);

to the presence of essential amino acids, minerals and vitamins, as well as favourable fatty acid composition (Klarić et al., 2018a). Similar to present study, Farag and El Rayes (2016) have examined the highest concentrations of pollen supplementation in broiler feed mixtures and obtained the highest values of average daily gain and the best feed conversion ratio in the group with 0.6% pollen. The improvement in the production performance of chickens as a consequence of the use of pollen in the diet is also confirmed by the study of Hosseini et al. (2016) who report significant differences in the production indicators of chickens fed mixtures with the addition of 2% of this feed compared to the control group (0%). In present study, no significant differences in chicken mortality were found between the experimental groups and the control, where the obtained values were within the technological norms of the tested hybrid. Contrary to our results, Klarić et al. (2018a) report lower mortality values for chickens fed mixtures with pollen and propolis, individually and in combination, compared to the control. Certain discrepancies in the obtained production performance results between studies can be explained by differences in the chemical composition of the pollen used, which mostly depends on the plants from which the bees collect it (Oliveira et al., 2013; Taha, 2015).

The effects of bee pollen powder consumption on the results of microbiological analysis of the cecum content of chickens are shown in Table 3. It was found that the total aerobic bacteria count did not differ significantly between the groups. The least aerobic bacteria were isolated in the cecum of chickens of group P1.0. Also, the *Escherichia coli* count did not differ under the influence of the examined factor, the highest values were determined in group C and the lowest in P1.0. Significant differences ($p < 0.01$) were found for the *Enterobacteriaceae* count where a higher number was found in groups C and P0.25 compared to groups P0.75 and P1.0. In mixtures in which pollen was added, it was found that the *Enterobacteriaceae* count gradually decreased with increasing concentration.

Table 3. Microbiological analysis of cecum content of broilers fed 42 days with different levels of supplemented bee pollen in feed, log10 CFU/g
Mikrobiologische Analyse des Blinddarminhalts von Broilern, die 42 Tage lang mit unterschiedlich hohen Zugaben an Bienenpollen im Futter gefüttert wurden, log10 KBE/g

	Treatments						
	C	P0.25	P0.5	P0.75	P1.0	SEM	p
Total number of aerobic bacteria	8.25 ± 0.49	8.39 ± 1.59	8.36 ± 0.77	8.26 ± 0.47	8.28 ± 0.50	0.109	0.993
Enterobacteriaceae	7.90^{ab} ± 0.14	8.11^{a} ± 0.30	7.76^{bc} ± 0.52	7.62^{c} ± 0.17	7.51^{c} ± 0.54	0.054	0.002
Escherichia coli	6.99 ± 1.45	6.73 ± 0.84	6.88 ± 0.40	6.77 ± 0.48	6.20 ± 1.02	0.120	0.277

Values are given as mean ± SD (standard deviation), n = 12 chickens per group; SEM, Standard error of the means; $^{a, b, c}$ In a row, the least squares means with a different superscript differ significantly ($p < 0.05$)

Pollen contains various types of nutrients and bioactive substances that can promote growth and protect the health of the intestinal tract (Liu et al., 2010). Poultry intestines are inhabited by populations of microorganisms that affect the digestion of food, the utilization of energy from certain nutrients and thus the health of the host. Basim et al. (2006) have concluded that, with the use of pollen, the population of harmful microorganisms can be reduced thanks to its antibacterial activity. The results obtained in our study showed a significant ($p < 0.01$) decrease in the *Enterobacteriaceae* count with an increase in the concentration of bee pollen in the mixture and can be compared with the study of Kročko et al. (2012) who report significantly better results in terms of the total *Enterobacteriaceae* count as a result of the use of pollen in the diet of broiler chickens compared to the control. Similar results were obtained by Kačaniova et al. (2013) who have recorded the highest *Enterobacteriaceae* count in the control group. In contrast to present study, the authors used pollen extract in a concentration of 0.5 to 4.5% in the mixture.

The results of slaughter performance of broiler chickens fed mixtures with the addition of different pollen concentrations are shown in Table 4. Slaughter yield, share of breasts, drumsticks, thighs, wings, adominal fat, heart, liver and gizzard did not differ significantly under the influence of the examined factor.

The increase in pollen concentration in chicken feed rations did not affect significant differences in any of the examined carcass quality parameters. In accordance with our results are study results obtained by Haščik et al. (2020) showing no differences in chicken slaughter yield and breast and leg shares as a consequence of the use of pollen extract in combination with probiotics. Also, similar to the results obtained in our study. Abood and Ezzat (2018) have not determined the significance of the effect of different concentrations of pollen added on the shares of abdominal fat, liver and heart, while contrary to our results, the authors state significant differences in yield values between experimental groups and control. Farag and El Rayes (2016) state that the use of pollen as a dietary supplement in chickens has no significant effect on the share of gizzard and liver, but shows significant effect on the slaughter yield.

Conclusions

Based on the results obtained in this study, it can be concluded that the addition of 0.75% of finely ground bee pollen to the complete feed mixture for broilers from the first day of fattening has positive effects on average growth and feed conversion ratio at the end of production, as well as more favourable microbiological composition of cecum. Further increase of pollen concentration in the mixture showed no positive effects.

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Table 4. Effects of different levels of addition of bee pollen to broiler diet on slaughter traits of broiler chickens
Auswirkungen unterschiedlich hoher Zugaben von Bienenpollen zum Broilerfutter auf Schlachtleistungsmerkmale

	Treatments						
	C	P0.25	P0.5	P0.75	P1.0	SEM	p
Body weight, g	2583.9 ± 219.0	2647.5 ± 310.7	2694.2 ± 371.5	2674.2 ± 319.8	2686.5 ± 318.5	39.214	0.911
Carcass weight, % BW	69.54 ± 1.65	69.47 ± 1.57	70.17 ± 1.90	70.79 ± 1.60	70.24 ± 1.35	0.212	0.264
Breasts, % CW	29.40 ± 2.14	29.37 ± 2.19	30.20 ± 1.91	30.69 ± 1.69	30.55 ± 1.63	0.251	0.297
Drumsticks, % CW	17.16 ± 0.69	17.31 ± 0.82	17.82 ± 0.58	17.76 ± 0.64	17.84 ± 1.01	0.102	0.092
Thigh, % CW	15.19 ± 1.01	15.70 ± 0.62	15.29 ± 1.11	14.86 ± 0.93	15.14 ± 0.79	0.119	0.269
Wings, % CW	11.10 ± 0.57	10.69 ± 0.69	10.49 ± 0.66	10.35 ± 0.66	10.63 ± 0.60	0.086	0.063
Abdominal fat, % CW	0.85 ± 0.40	0.73 ± 0.39	0.82 ± 0.51	0.62 ± 0.31	0.61 ± 0.36	0.051	0.464
Organ weights, % BW							
Heart	0.57 ± 0.05	0.56 ± 0.07	0.54 ± 0.06	0.57 ± 0.06	0.54 ± 0.05	0.007	0.668
Liver	1.97 ± 0.13	1.99 ± 0.17	1.90 ± 0.17	1.88 ± 0.15	1.97 ± 0.13	0.020	0.250
Gizzard	1.80 ± 0.12	1.78 ± 0.16	1.80 ± 0.13	1.75 ± 0.15	1.77 ± 0.19	0.019	0.923

Values are given as mean ± SD (standard deviation), n = 12 chickens per group; SEM: Standard error of the means; BW: Body weight; CW: Carcass weight

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