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EFFECT OF *BOSWELLIA SERRATA* DIETARY SUPPLEMENTATION ON GROWTH PERFORMANCE, GASTROINTESTINAL MICROFLORA, AND MORPHOLOGY OF BROILERS*

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Abstract

The study aimed to determine the effect of three different levels of *Boswellia serrata* resin added to broiler diets on the fundamental production parameters, dry matter digestibility, organic matter digestibility, energy digestibility, microbiological condition of the gastrointestinal tract, and histomorphology of the walls of the small intestine. Two hundred Ross 308 chicks were assigned into 4 groups (50 birds of equal body weight) in 5 replications of 10 chicks each (5 females and 5 males). The experiment lasted 6 weeks. The control group (B_0) was fed a standard mixture, without supplementation, whereas in groups $B_{1.5,}$ B_2, and $B_{2.5,}$ the *Boswellia serrata* resin was added at the levels of 1.5, 2.0, and 2.5% of the complete feed mixture, respectively. The dietary supplementation with 2.0 and 2.5% of *Boswellia serrata* resin contributed to a significant increase in the length of the duodenum and total intestine and in the digestibility of dry matter and organic matter in feed. In these groups, the values of FCR and EEI were positively influenced ($P<0.05$) and an improvement in the structure of the jejunal wall was also recorded ($P<0.05$). An increase in the count of *Lactobacillus* and *Enterococcus* in the intestinal contents in broilers fed with the *Boswellia serrata* resin supplemented diets was observed. In conclusion, the *Boswellia serrata* resin can be considered as an effective feed additive, which stimulates production and has a positive effect on intestinal microflora and morphology of broilers.

Key words: *Boswellia serrata*, broiler, production performance, intestine, digestibility, resin

Phytogenic feed additives such as herbs or spices have become increasingly popular in poultry production. With regard to the content of biologically active substances, they have a multidimensional effect leading to improvement in the health status of birds and to increased rearing efficiency (Abdel-Wareth et al., 2012; Cho et al., 2014).

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Boswellia serrata resin is included in the group of feed additives approved for use in poultry production according to the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (EURFA, 2015). The resin of *Boswellia* species originates from India, Pakistan, Africa, and the Arabian Peninsula, where it is obtained from trees of the *Burseraceae* family. It is also known as frankincense, olibanum, salai guggal, loban, or kundur (Thulin and Warfa, 1987; Afsharypuor and Rahmany, 2005) and is famous as a traditional medicine of the East, since it appears to possess anti-inflammatory, antiseptic, and even anticancer or anxiolytic properties. Bark, leaves, flowers, and resin obtained by incision of the trunk or branch are used in treatment. After drying, the resin forms semi-transparent up to 3-cm chunks (Khare, 2004). Currently, phytomedicines containing active substances derived from *Boswellia* spp. are available on the market. An example is Swanson Boswellia registered under the Swanson trademark. It contains 400 mg of *Boswellia serrata* (whole herb) in a capsule. The recommended daily dose is 800 mg. It exhibits anti-inflammatory activity and is recommended in inflammation and osteoarthritis treatment (www.swansonvitamins.com). 5-Loxin® is another supplement available on the market. It contains a *Boswellia serrata* extract with a higher concentration of AKBA, standardized to 30% (US 20040073060 A1 – Patent publication).

These therapeutic properties are associated with the presence of many biologically active substances. The main component of frankincense resin is its oil (60%). It contains mono- (13%) and diterpenes (40%) as well as ethyl acetate (21.4%), octyl acetate (13.4%), and methylanisole (7.6%). The main biological activity among terpenes is attributed to 11-keto-ß-acetyl-beta-boswellic acid (KBA), acetyl-11-keto-ß-boswellic acid (AKBA), and acetyl-α-boswellic acid (AαBA) (Camarda et al., 2007). The therapeutic effects of *Boswellia* have been confirmed by numerous scientific studies. In their investigations conducted in an orthotopic mouse model, Yadav et al. (2012) reported significant inhibition of CRC (colorectal cancer) growth at a dose of AKBA of 50 mg/kg body weight. In another murine-mode study, significant reduction of inflammatory cytokine and blood glucose levels was reported after a 10-day treatment with KBA and AKBA (7.5 to 15.0 mg/kg) (Shehata et. al., 2015). Similar results were obtained by Schrott et al. (2014) in a human study. In rats with collagen-induced arthritis, a positive effect of the *Boswellia serrata* extract (at the doses of 100 and 200 mg/kg body weight administered once a day for 21 days) on inflammatory mediators and oxidative stress was observed (Umar et al., 2014). Catanzaro et al. (2015) investigated the mechanism of the pharmacological potential of *Boswellia serrata* in colonic epithelial cell monolayers in an *in vitro* experimental model of intestinal inflammation. A positive correlation was found between the antioxidant activity and sustenance of the integrity and activity of intestinal epithelium. However, there is no information about its adequacy for use in poultry and the effect of its supplementation on production parameters and health status in birds. Therefore, the purpose of the present study is to determine the effect of three different levels of *Boswellia serrata* resin supplemented to the diets of broiler chickens on the fundamental production parameters, dry matter digestibility, organic matter digestibility, energy digestibility, microbiological condition of the gastrointestinal tract, and histomorphology of the walls of the small intestine.

Material and methods

Experimental design, birds, and diets

Two hundred one-day-old Ross 308 chicks were allocated into 4 groups (50 birds of equal body weight) in 5 replications of 10 chicks each (5 females and 5 males). The experiment lasted 6 weeks and each replication was housed in cages with an area of 1 m^2, with *ad libitum* access to feed and water, under controlled temperature and humidity conditions. Three days before the arrival of the chickens, the floor was heated to 29°C, litter to 30°C, and air to 32°C, at 63% relative humidity. Such thermal conditions were maintained up to 4 days after the placement of the chickens, and then the air temperature was gradually reduced to 20°C. The lighting regime was adjusted according to the guidelines of the Ross 308 broilers Management Handbook (Aviagen, 2014).

The experiment was carried out after approval by the Second Local Ethics Committee at the University of Life Sciences in Lublin (No. 27/2014, No. 34/2015).

The basal feed mixtures were made from cereal meal middlings (wheat and corn) and post-extraction soybean meal as recommended by the Ross 308 broiler Nutrition Specifications (Aviagen, 2013) (Table 1). The chickens were fed with three types of mixtures: starter – from day 1 to 21, grower – from day 22 to 35, and finisher – from day 36 to 42 of rearing. The starter mixture was administered to chicks in a crumbled form, and the grower and finisher mixtures in a granulated form. The control group (B_0) was fed a standard resin-free mixture. In groups $B_{1.5,}$ $B_{2.0}$, and $B_{2.5,}$ frankincense was added at the level of 1.5, 2.0, and 2.5% of the complete feed mixture, respectively. The *Boswellia serrata* resin added to the mixtures contained 95.34% of dry matter, 1.59% d.m. of ash, 2.65% d.m. of protein, 63.88% d.m. of fat, and 2.38 acetyl-11-keto-ß-boswellic acid (AKBA). The diets of the four experimental groups were isoenergetic and isoprotein.

Growth performance and feed digestibility

The values of body weights and feed intake of each broiler chicken were recorded at 1, 10, 21, 35, and 42 days of age. Body weight gain and feed conversion ratio (FCR) values were calculated for each period. Mortality rates were recorded daily, and the weights of dead birds were used to adjust average daily gain, average daily feed intake, and FCR.

Feed digestibility was evaluated by the method described by Kussaibat and Leclercq (1985) on 64 birds – 16 from each experimental group (in 4 replications of 4 birds) at the final stage (36–42 day of age). In the collected excreta, the content of dry matter and organic matter was determined (AOAC, 2000), and the content of nitrogen was determined according to Ekman et al. (1949). The dry matter and organic matter digestibility ratios and the content of nitrogen-corrected metabolizable energy (ME_N) were calculated for each mixture according to formulas given by the European Table of Energy Values for Poultry Feedstuffs (1986).

Table 1. Ingredient and nutrient composition of diets

Item	Diets											
	S (1–21 days)[1]				G (22–35 days)[2]				F (36–42 days)[3]			
	B_0	$B_{1.5}$	B_2	$B_{2.5}$	B_0	$B_{1.5}$	B_2	$B_{2.5}$	B_0	$B_{1.5}$	B_2	$B_{2.5}$
Ingredients (%)												
maize	30	30	30	30	29	29	29	29	30	30	30	30
wheat	20.0	20.0	20.0	20.0	23.0	23.0	23.0	23.0	26.0	26.0	26.0	26.0
soybean meal (460 g/kg CP)	39.47	39.47	38.97	38.47	36.76	37.26	36.76	36.26	32.13	32.13	31.63	31.13
Boswellia serrata	-	1.5	2.0	2.5	-	1.5	2.0	2.5	-	1.5	2.0	2.5
soybean oil	6.0	4.5	4.5	4.5	7.0	5.0	5.0	5.0	8	6.5	6.5	6.5
phosphate 2-Ca	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
limestone	1.2	1.2	1.2	1.2	1.0	1.0	1.0	1.0	0.7	0.7	0.7	0.7
NaCl	0.33	0.33	0.33	0.33	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-Methionine[a]	0.36	0.36	0.36	0.36	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
L-Lysine[b]	0.34	0.34	0.34	0.34	0.36	0.36	0.36	0.36	0.34	0.34	0.34	0.34
vitamin-mineral premix[c]	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.2	0.2	0.2	0.2
Calculated analysis												
AME (MJ/kg)	12.55	12.43	12.43	12.44	12.97	12.90	12.93	12.95	13.39	13.33	13.36	13.37
CP (g/kg)	212.0	211.0	212.0	212.0	192.0	192.0	193.0	193.0	185.0	185.0	185.0	185.0
Lys (g/kg)	13.8	13.8	13.8	13.8	12.9	12.9	12.9	12.9	11.3	11.3	11.3	11.3
Met + Cys (g/kg)	10.5	10.5	10.5	10.5	9.8	9.8	9.8	9.8	9.0	9.0	9.0	9.0
Ca (g/kg)	9.6	9.6	9.6	9.6	8.7	8.7	8.7	8.7	7.9	7.9	7.9	7.9
Available P (g/kg)	4.8	4.8	4.8	4.8	4.3	4.3	4.3	4.3	3.9	3.9	3.9	3.9
Na (g/kg)	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7

[1]S – starter mixture; [2]G – grower mixture; [1]F – finisher mixture.

[a] MetAMINO®. Evonik Degussa GmbH. Essen. Germany. 990 g/kg of methionine.

[b] Ajinomoto Eurolysine S.A.S. Amiens. France. 780 g/kg lysine.

[c] Added minerals and vitamins per kg:

Starter mixture – Mn 100 mg, I 1 mg, Fe 40 mg, Zn 100 mg, Se 0.15 mg, Cu 10 mg, Vit. A 15 000 IU, Vit. D_3 5 000 UI, Vit. E 75 mg, Vit. K_3 4 mg, Vit. B_1 3 mg, Vit. B_2 8 mg, Vit. B_6 5 mg, Vit. B_{12} 0.016 mg, biotin 0.2 mg, folic acid 2 mg, nicotic acid 60 mg, pantothenic acid 18 mg, choline 1 800 mg.

Grower mixture – Mn 100 mg, I 1 mg, Fe 40 mg, Zn 100 mg, Se 0.15 mg, Cu 10 mg, Vit. A 12 000 IU, Vit. D_3 5 000 UI, Vit. E 50 mg, Vit. K_3 3 mg, Vit. B_1 2 mg, Vit. B_2 6 mg, Vit. B_6 4 mg, Vit. B_{12} 0.016 mg, biotin 0.2 mg, folic acid 1.75 mg, nicotic acid 60 mg, pantothenic acid 18 mg, choline 1 600 mg.

Finisher mixture – Mn 100 mg, I 1 mg. Fe 40 mg, Zn 100 mg, Se 0.15 mg, Cu 10 mg, Vit. A 12 000 IU, Vit. D_3 5 000 UI, Vit. E 50 mg, Vit. K_3 2 mg, Vit. B_1 2 mg, Vit. B_2 5 mg, Vit. B_6 3 mg, Vit. B_{12} 0.011 mg, biotin 0.05 mg, folic acid 1.5 mg, nicotic acid 35 mg, pantothenic acid 18 mg, choline 1 600 mg.

Sample collection and chemical analyses, histomorphological measurements of the small intestine, and microbiological examinations of intestinal digesta

Twenty birds (10 females and 10 males) with an average body weight of each group were selected and slaughtered by decapitation. Dissection analysis was carried out according to Ziołecki and Doruchowski (1989), during which the gastrointestinal tract was separated and the weights of the proventriculus and the gizzard were determined. The lengths of the duodenum and jejunum and the total intestinal length were also measured.

Samples of the intestines after separation were fixed in Bouin's solution (Gabe, 1976), dehydrated, and paraffin-embedded. Longitudinal, 6-7 µm thick, consecutive paraffin sections were prepared and stained with hematoxylin–eosin (HE) (Zawistowski, 1986). Morphometric analyses were performed on the duodenum and jejunum of 8 broilers per treatment. The preparations were analyzed under a light microscope Axio Imager (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). Patches with a correct structure were scanned with a Mirax Desk scanner (Carl Zeiss Microscopy GmbH, Jena, Germany). The measurements of the intestinal structures were performed using the Zeiss Axiovision LE image analysis program, ver. 4.1. (Zeiss MicroImaging GmbH, Jena, Germany). The height of the mucous layer, villus height, and crypt depth were measured, and the villus height to crypt depth ratio was then calculated. Five measurements of each structure were performed per sample. The villus height was measured from the tip of the villus to the villus-crypt junction, whereas the crypt depth was defined as the depth of the invagination between adjacent villi.

The samples of the small intestine collected during the dissection were weighed, diluted with phosphate buffered saline (PBS), and homogenized (Stomacher Bag-Mixer 400, Interscience, the Netherlands). Then, the supernatant samples were fixed for 16 hours at 4°C in paraformaldehyde (Sigma) (Fuchs et al., 2007). The resultant suspension was homogenized again with glass balls (3 mm) for 5 minutes (Merck, Germany). By means of centrifugation (5000 x g/5 min.), eukaryotic cells and undigested food were removed (MPW-350R, MPW Medical Instruments, Warsaw, Poland). The resultant supernatant was placed on white polycarbonate membrane filters (pore size – 0.2 µm) (Millipore, Ireland) by means of sterile filter units (Nalgene®, USA). Samples for further analyses were stored at a temperature of –20°C.

Using the fluorescent *in situ* hybridization method, the microorganisms were determined using group-specific probes (Thermo Fisher Scientific, Ulm, Germany): Eub338 5' -GCTGCCTCCCGTAGGAGT-3' for the *Bacteria* domain, Lab151 5' GGTATTAGCA / TCTGTTTCCA for *Lactobacillus-Enterococcus*, Bif164 5' – CATCCGGCATTACCACCC for *Bifidobacterium* sp., Sal 5'-TGCGGTTATTAAC-CACAACA-3' for *Salmonella* sp., Chis150 5'-TTATGCGGTATTAATCTYCCTTT for *Clostridiaceace*, and Non- Eub338 5' -CGACGGAGGGCATCCTCA-3' as a negative control.

The total count of *Lactobacillus* and *Enterococcus* and *Bifidobacterium* was identified using lysozyme diluted in TE-His buffer (100 mM Tris-HCl [pH 8.0], 50 mM EDTA) placed on a membrane filter. After incubation for 10 minutes at room temperature, the filters were dried and dehydrated in ethanol. 10 µL of the hybridization

buffer (5 M NaCl, 20 mM Tris/HCl, formamide in respective concentrations, distilled water, 0.01% SDS) with indocarbocyanine (Cy3) at a concentration of 50 ng/μL were placed on the filters. Depending on the type of bacteria identified, hybridization was carried out for 90 minutes at a temperature of 46°C or for 24h at 35°C. The remains were flushed with the buffer (900 mM NaCl, 20 mM Tris/HCl, 5 mM EDTA, distilled water, 0.01% SDS) in a water bath at a temperature of 48°C or 37°C for Gram-positive bacteria. The samples were flushed with distilled water, dried at room temperature, stained with DAPI at a concentration of 1 μg/ml (Porter and Feig, 1980), and covered with Citifluor and Vectashield immersion oils (4:1). The samples were covered with slips and stored at –20°C for further microscopic analyses. The stained preparations were analyzed using an epifluorescent microscope (Olympus BX51, Olympus Optical Co. Ltd., Tokyo, Japan) by means of filters (360–370 nm filter BP, DM excitation 400 nm, 420 nm) BA and Olympus Cell F software.

Statistical analysis

The data were analyzed by ANOVA using one-way analysis of variance (α= 95 and 99%; $P<0.05$ and $P<0.01$), test F of equal variance and calculating the mean values for the groups ($\bar{x}$), and standard error of the mean (SEM). The significance of differences between the mean values of the analyzed characteristics was determined using Duncan's test (post-hoc) by means of Statistica 10.0 software. Linear regression equations allowing estimation of the experimental (productivity, histomorphological, and microbiological) results were calculated and determination coefficients for regression (R^2) were specified. The direction and intensity of the relationships between the level of *Boswellia serrata* addition and the productivity, histomorphological, and microbiological parameters were determined by means of Spearman's rank correlation coefficients (r_s).

Results

Growth performance and feed digestibility

In the present study, broilers consumed similar amounts of feed, regardless of the type of ration, although a certain trend to reduce the total intake of feed was observed in chickens from groups $B_{1.5}$, $B_{2.0}$, and $B_{2.5}$ ($R^2 = 0.466$) with reference to birds fed with the control diet (Table 2). Daily BWG had similar values among the experimental groups. However, lower FCR and higher EEI values ($P<0.05$) were observed in birds from groups $B_{2.0}$ and $B_{2.5}$ compared to B_0. No differences in mortality rates were found, with only one dead chicken in group $B_{1.5}$ throughout the experimental period.

The supplementation of broiler diets with *Boswellia serrata* resulted in changes in the development and functioning of their gastrointestinal tract. Higher values for duodenum ($P<0.05$) and total intestinal length ($P<0.01$) were found in broilers from $B_{2.0}$ and $B_{2.5}$ compared to groups B_0 and $B_{1.5}$. A strong positive correlation was recorded between the length of the duodenum and total intestinal length and the level of *Boswellia serrata* supplementation of the mixtures for chickens (respectively

r_s = 0.815; P=0.048 and 0.835; P=0.037). On the other hand, the longest jejunum (P<0.001) was characteristic of birds from group $B_{1.5}$. The length of this section of the gastrointestinal tract was negatively linearly correlated with the level of *Boswellia serrata* resin supplementation in the diet (r_s = –0.699; P=0.046). The above results were reflected in the higher dry matter and organic matter digestibility (P<0.01) in birds from groups $B_{2.0}$ and $B_{2.5}$ characterized by longer intestines.

Table 2. Efficiency of broiler feeding during the whole fattening period (1–42 days) *

Item	Treatment**				Statistical parameters	
	B_0	$B_{1.5}$	$B_{2.0}$	$B_{2.5}$	SEM	P-value
Feed intake (g/kg)						
daily	102.9	102.3	100.7	101.8	1.45	0.095
total	4221	4195	4128	4174	19.45	0.078
Daily BWG (g/bird)	56	53	54	55	0.18	0.278
BW (g)						
initial	43.0	42.8	43.1	42.7	1.74	0.104
final	2526	2515	2547	2560	15.47	0.128
FCR (kg/kg)	1.73 a	1.67 ab	1.62 b	1.63 b	0.03	0.038
Mortality of chickens (head)	0	1	0	0	–	–
EEI	356 b	364 b	388 ab	397 a	2.45	0.026

*The data represents mean values of each broiler per treatment (n=50). **B_0 $B_{1.5}$, B_2, $B_{2.5}$ – dietary supplementation with *Boswellia serrata* at the level of 0% (control group), 1.5%, 2%, and 2.5%; respectively. a, b, c – means with no common letters are significantly different (P<0.05).

Table 3. Condition of the gastrointestinal tract of 42-day-old broilers fed mixtures supplemented with *Boswellia serrata* *

Item	Treatment**				Statistics	
	B_0	$B_{1.5}$	$B_{2.0}$	$B_{2.5}$	SEM	P-value
Metabolic body weight (MBW) (kg)	0.356	0.355	0.359	0.360	0.047	0.157
Proventriculus						
weight (g/kg MBW)	4.423	4.526	4.492	4.228	0.061	0.107
share in MBW (%)	1.241	1.273	1.252	1.173	0.023	0.073
Gizzard						
weight (g/kg MBW)	22.51	22.67	22.37	22.18	0.487	0.451
share in MBW (%)	6.318	6.383	6.239	6.163	0.064	0.321
The length of intestines (cm/kg MBW)						
duodenum	20.81 b	20.24 b	22.45 a	22.49 a	0.645	0.029
jejunum	55.91 AB	56.38 A	54.67 B	54.24 B	1.65	<0.001
total length of intestines	164.5 B	167.4 B	173.1 A	170.2 AB	4.16	<0.001
Digestibility*** (%)						
dry matter	80.45 B	80.66 B	83.79 A	84.89 A	5.47	<0.001
organic matter	80.15 B	83.78 AB	84.98 A	85.14 A	4.98	<0.001
gross energy	79.84	79.68	79.94	80.34	6.48	0.348

*The data represents mean values of 20 broilers per treatment; **B_0, $B_{1.5}$, B_2, $B_{2.5}$ – dietary supplementation with *Boswellia serrata* at the level of 0% (control group), 1.5%, 2%, and 2.5%; respectively; *** The data represents mean values of 16 broilers per treatment; a,b,c – means with no common letters are significantly different (P<0.05); A, B, C – means with no common letters are significantly different (P<0.01).

Intestinal microbiology

Dietary supplementation with the *Boswellia serrata* resin significantly affected the intestinal microbiology in the broilers (Table 4). Dietary incorporation of frankincense at the level of 2.5% increased the bacterial count in the broilers' intestines ($P<0.05$), especially that of *Lactobacillus* and *Enterococcus* ($P<0.05$). On the other hand, dietary supplementation at the levels of 1.5 and 2.0% did not have any effect on the total count of the examined bacteria. The population of *Bifidobacterium* and *Clostridiaceae* remained at the same level among the experimental groups. Finally, no *Salmonella* sp. bacteria were found.

Table 4. Bacteria identified in the intestinal contents of broiler chickens aged 42 days (bacterial count per g of intestinal contents [$\log_{10}$])*

Item	Treatment**				Statistics	
	B_0	$B_{1.5}$	$B_{2.0}$	$B_{2.5}$	SEM	P-value
Total count	9.65 a	8.72 b	9.14 ab	9.64 a	0.278	0.043
Bacteria	8.57 b	8.49 b	8.97 ab	9.25 a	0.089	0.026
Lactobacillus and *Enterococcus*	7.79 b	8.02 ab	8.41 ab	8.56 a	0.067	0.037
Bifidobacterium	8.12	8.16	8.33	8.46	0.048	0.108
Salmonella sp.	ND	ND	ND	ND	-	-
Clostridiaceae	7.45	7.41	7.16	7.04	0.081	0.348

*The data represents mean values of 8 replications per treatment. **B_0 $B_{1.5}$, B_2, $B_{2.5}$ – dietary supplementation with *Boswellia serrata* at the level of 0% (control group), 1.5%, 2%, and 2.5%; respectively. a,b,c – means with no common letters are significantly different ($P<0.05$).

Histomorphological measurements of the small intestine

The addition of frankincense resin in the diets of broilers was found to have a significant effect only on the structure of the chickens' jejunal walls (Table 5). In groups $B_{2.0}$ and $B_{2.5}$, a significant reduction in the crypt depth was recorded in the histological preparations of the jejunum (on average by about 15%) along with an increase in the villus:crypt ratio (by about 19%) compared to the control group (B_0) ($P<0.05$). The magnitude of the frankincense effect on the morphology of the jejunal mucosa is illustrated by Spearman's rank correlation coefficients: r_s=–0.583 and 0.648 for the crypt depth and the villus:crypt ratio, respectively. On the other hand, the increased values of regression determination coefficients for these variables (R^2=0.874 and 0.856, respectively) revealed an efficient adjustment of the equations to the results obtained. The observed changes are correlated with the decreased length of the birds' jejunum in these groups ($B_{2.0}$ and $B_{2.5}$) (Table 3), the increased count of desirable *Lactobacillus* and *Enteroccocus* bacteria (Table 4), and the improved dry matter and organic matter digestive efficiency (Table 3). This was also reflected in improved FCR recorded in these groups (Table 2). The dietary supplementation with *Boswellia serrata* did not have a significant impact on the thickness of the mucosa and the height of the villi in the small intestine as well as the depth of crypts in the duodenum (Table 5).

Table 5. Effect of *Boswellia serrata* supplementation on the histomorphology of the small intestine of a broiler chicken (µm)*

	Treatment**				Statistics	
	B_0	$B_{1.5}$	$B_{2.0}$	$B_{2.5}$	SEM	P-value
Duodenum						
mucosa thickness	2150.58	2215.05	2279.33	2195.29	92.900	0.779
crypt depth	355.81	361.48	367.14	351.03	12.533	0.681
villus height	1992.80	2002.40	2079.20	2083.40	81.280	0.776
villus:crypt ratio***	5.65	5.55	5.83	5.93	0.270	0.671
Jejunum						
mucosa thickness	2081.65	2052.59	2023.15	1906.23	72.035	0.877
crypt depth	374.66 a	346.68 ab	318.34 b	320.07 b	10.742	0.021
villus height	1802.38	1800.48	1798.69	1818.05	13.950	0.871
villus:crypt ratio***	4.81 b	5.25 ab	5.74 a	5.68 a	0.178	0.038

*The data represents mean values of 8 replications per treatment; **B_0, $B_{1.5}$, B_2, $B_{2.5}$ – dietary supplementation with *Boswellia serrata* at the level of 0% (control group), 1.5%, 2%, and 2.5%; respectively; %. *** Villus:crypt ratio – villi length ratio to the depth of the crypts. a, b, c – means with no common letters are significantly different ($P<0.05$).

Discussion

The use of the *Boswellia serrata* resin at the level of 2 and 2.5% in mixtures for broilers caused a negative trend in the feed intake throughout the rearing period, resulting in improved FCR and EEI values ($P<0.05$). The use of phytogenic feed additives and, in particular, therapeutic plants in animal production, improves production efficiency. The introduction of feed materials rich not only in basic nutrients but also in specific bioactive substances enhances the birds' health condition, which determines their high productivity (Hashemi and Davoodi, 2011). This phenomenon can also be observed upon the application of traditional European herbs and spices (Florou-Paneri et al., 2006; Christaki et al., 2012) or even fungi (Giannenas et al., 2010 a, 2010 b, 2011) as well as phytobiotics (herbs, mushrooms, resins) cultivated and collected outside Europe (Landy et al., 2011; Nadeem, 2012). However, excessive doses of phytobiotics with intense properties such as the *Boswellia serrata* resin can inhibit animal growth. Singh et al. (2008) found that a dose of pure *Boswellia serrata* at the level of 500 mg/kg of body weight of rats was sufficient to inhibit their growth. Such a phenomenon was not observed in the present study, which suggests that the doses of the frankincense supplement used did not have a negative effect on the growth of the efficient adjustment of the equations with the broilers results.

The supplementation at the levels of 2.0 and 2.5% with the *Boswellia serrata* resin contributed to an increase in the digestibility of dry matter and organic matter. The intensity of digestion of feed nutrients is different in the respective sections of the gastrointestinal tract. It is determined, among other things, by the size of the proventriculus and the gizzard, the level of secretion of hydrochloric acid and pepsinogen, and the frequency of exchange of the digesta between the proventriculus

and the gizzard. Although no effect of the *Boswellia serrata* supplement on the size of these avian internal organs was observed in the present study, the higher digestive efficiency can be associated with the processes occurring in these organs such as dissolution of mineral salts, mainly Ca and P (Jamroz et al., 2006). Gupta et al. (2001) confirmed that the dietary supplementation with the *Boswellia serrata* resin increases the absorption of iron, calcium, and phosphorus, which can also contribute to improved digestibility of dry matter and organic matter in feed. The results of the present study indicate that the final efficiency of nutrient digestion is largely affected by the length of intestines, their physical condition, and microbiological environment. The supplementation at the levels of 2.0 and 2.5% with the *Boswellia serrata* resin contributed to a significant increase in the length of the duodenum and total intestine. The small intestine of birds plays a significant role in digestion and absorption of nutrients. Liver enzymes emulsifying fats and pancreatic enzymes hydrolyzing fats (pancreatic lipase), carbohydrates (pancreatic alpha–amylase), and proteins (trypsin and chymotrypsin) are secreted into the duodenum (Adil et al., 2010; Shih and Hsu, 2006). Qurishi et al. (2010) observed that boswellic acid stimulates secretion of pancreatic enzymes leading to an improvement of protein and energy digestibility, a reduction of endogenous losses of nitrogen and ammonia, and production of other microbial metabolites. On the other hand, Dzubak et al. (2006) reported a stimulating effect of boswellic acid on liver function. Moreover, Borrelli et al. (2006) showed that supplementation of feed with the *Boswellia serrata* resin normalizes intestinal peristalsis, reduces the pH of the digesta, improves passage of the digesta, has a trophic effect on the mucosa of the gastrointestinal tract, and prevents diarrhea in mice and guinea pigs. Krieglstein et al. (2002) further confirms that *Boswellia* sp. resins appear to stimulate digestive functions, reduce gases, and enhance the flow of digestive juices. As demonstrated in the present study, the optimum conditions for dry matter and organic matter digestion in the gastrointestinal tract appeared in broiler chickens fed mixtures with the highest amount of *Boswellia serrata* resin added (B_2 and $B_{2.5}$).

Many researchers report strong antibacterial properties of *Boswellia serrata* resin due to the presence of verticilla-4 (20), 7,11-triene, incensol, acetyl-keto-boswellic acid, and α-, β-BA 3-oxo-tirucallic acids (Mothana et al., 2011). A strong antibacterial effect of frankincense was observed after application thereof in various forms and doses: as a water-based extract against *Proteus aeruginosa* and *Proteus vulgaris* (Alsaba et al., 2011), as a petroleum ether-based extract against *Staphylococcus aureus* (Raja et al., 2011), and as an acetone-based extract against *Eschericha coli* and *Klebsiella pneumoniae* (Rajendra et al., 2013). The results of the present study are in agreement with the above findings. A high negative correlation was observed between the total bacterial count in the chicken intestines ($r_s = –0.766$) and strains that easily become virulent such as *Escherichia coli* (r_s=–0.868) and the level of supplementation with the *Boswellia serrata* resin. Patel and Patel (2014) confirmed using a disc diffusion method that the resin of *Boswellia serrata*, regardless of the form in which it is applied (extracts based on water, petroleum ether, acetone, and methanol), shows a high zone of inhibition against *Escherichia coli*. Similar results were also obtained by Bushra et al. (2012), who carried out an experiment with 35-day-old hen

broilers. Mikhaeil et al. (2003) reported an antibacterial effect of frankincense on the stabilization of the microflora and the reduction of pH values in the gastrointestinal tract. Such conditions facilitate the growth of *Lactobacillus* strains. The dietary supplementation with the *Boswellia serrata* resin, particularly at the levels of 2.0 and 2.5%, possibly led to stimulation of the growth of the colonies of these bacteria in the intestines of broiler chickens. On the other hand, no *Salmonella* strains were detected in the chicken intestines regardless of the diet used. The absence or the reduced count of these bacteria in the gastrointestinal tract could be a result of the reduced pH of digesta in the intestines and the strong antibacterial effect of frankincense against *Salmonella sp*. strains (Rajendra et al., 2013). The multidimensional factors that appear to influence the quality of bacterial flora in natural conditions in the intestines of broilers reveals the necessity of further detailed studies in this field.

The level of health status of the animals, and particularly the condition of their gastrointestinal tract, has a detrimental effect on performance parameters. Therefore, it is very important to use feed supplements with a multiple positive impact on the organism. Phytobiotics belong to this category, as, among others, they have a positive effect on the intestinal microflora, enhance the function of the entire gastrointestinal tract, and increase the integrity of the epithelial barrier of the intestines providing protection against pathogenic microflora (Jamroz et al., 2005; 2006; Windisch et al., 2009). The positive effect of supplements such as herbs, spices, plant extracts, or essential oils on the gastrointestinal tract and the production performance of animals is widely described in the literature (Jamroz et al., 2006; Abdel-Rahman et al., 2014; Debnath et al., 2014; Zeng et al., 2015). In the present study, such positive morphological changes were observed in the jejunal wall in groups $B_{2.0}$ and $B_{2.5}$ and it is likely that they were induced by alterations in the composition of the intestinal microflora. The findings of the present study are difficult to compare with those obtained by other authors due to the lack of scientific data regarding the effect of *Boswellia serrata* dietary supplementation on the morphology of the intestinal mucosa in poultry. Hartmann et al. (2014) observed a reduction of the inflammatory condition and swelling in the intestinal submucosa of rats and reduced damage of the epithelium lining the crypts after the use of *Boswellia serrata*. In the present study, a reduced depth of the crypts and a simultaneous increase in the villus:crypt ratio ($P<0.05$), without a significant impact on the villus height, was found in groups supplemented with 2% and 2.5% of *Boswellia serrata* resin. Intestinal crypts participate in internal and external secretion of water and ions and in cellular restoration and proliferation of the epithelium covering the villi, and the villi take part in digestion and absorption (Imondi and Bird, 1966; Potten, 1998). The shortening of the villi and deepening of the crypts can lead to poor absorption of nutrients, increased secretion in the gastrointestinal tract, and a reduced yield (Xu et al., 2003). An increase in the height of the intestinal villi and the villus:crypt ratio is directly correlated with an increase in the cellular turnover in the epithelium. A reduction of intestinal crypts indicates a decrease in the exchange of enterocytes and a lower requirement of tissue development (Pluske et al., 1996; Samanya and Yamauchi, 2002). An increase in the depth of crypts is an indicator of an increased rate of production of enterocytes and their upward migration in intestinal villi (Koldovsky et al., 1966; Al-Mukhtar et al., 1982).

Pluske et al. (1996) observed a negative relationship between the depth of intestinal crypts and the capacity of hydrolyzing lactose and sucrose in pigs. This finding was explained by an increase in the rate of migration of enterocytes at an accelerated cellular turnover, due to which more immature cells with reduced enzymatic activity covered the villi. The authors of the present study observed a higher digestive efficiency of dry matter ($P<0.01$), organic matter ($P<0.01$), and an upward trend in energy digestibility in groups $B_{2.0}$ and $B_{2.5}$, with significantly shallower intestinal crypts in the jejunum compared to the group of birds not receiving the *Boswellia serrata* supplement.

Conclusions

The dietary supplementation with 2 and 2.5% of *Boswellia serrata* had a positive impact on feed digestibility and on the microbiological status and the structure of the walls of the small intestine of broiler chickens, which led to improved production efficiency. The results provide new and valuable information concerning options for using the *Boswellia serrata* resin as a feed supplement in poultry production.

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