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EFFECT OF PHYTOGENIC ADDITIVES ON PERFORMANCE, MORPHOLOGY AND CAECAL MICROFLORA OF BROILER CHICKENS

M. Vukić-Vranješ[1], N. Tolimir[1], Đ. Vukmirović[2], R. Čolović[2], V. Stanaćev[3], P. Ikonić[2], S. Pavkov[4]

[1]Institute of Science Application in Agriculture, 68b Blvd. D. Stefana, [Belg]rade 11000, R.Serbia
[2]Institute of Food Technology, 1 Blvd. Cara Lazara, Novi Sad 21000, R.Serbia
[3] Perutnina Ptuj – Topiko a.d., Petefi brigade 2, Bačka Topola 24300, R. Serbia
[4]Institute Goša, Milana Rakića 35, Belgrade 11000, R. Serbia
Corresponding author: e-mail: marinavranjes@vinfeed.com
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Abstract: The objective of the research is to investigate effects of phytogenic additives as broiler feed supplements on production and slaughtering performances, morphological parameters and caecal microflora of fattening chickens of Ross 308 provenance. The research was based on two trials. In both trials, two feeding treatments were studied: a control diet with no supplemental feed additives (K) and a diet with a phytogenic additive supplemented to the control diet (1000g/t of Biomin PEP 1000, during the whole period). In the first trial, a total of 304 chickens were studied, in two treatments with four repetitions per pen with 38 chickens. The second trial included 16800 chickens, and it was conducted as a macro trial, that is, a pen with 8400 chickens was the subject of the trial. The results have shown that the chickens fed with the supplemental phytogenic additive have achieved significantly more favourable feed conversion rate. Differences in body mass and mortality rate between the trial group and the control were not statistically significant. Phytogenic additives did not have significant effects on breast portion and abdominal fat. Adding the phytogenic additive did not have significant effects on morphological parameters, but there was a tendency to increasing villous height and crypt depth. In both trials, the chicken groups fed with the supplemental phytogenic additive had less of all investigated groups of bacteria, and the differences were statistically significant for *Enterobacteriaceae* and *Staphylococcus* in the first, and *Enterobacteriaceae* and *Enterococcus* in the second trial. It can be concluded that the diet with the supplemental phytogenic additive had a positive effect on broiler performances, jejunum morphology parameters and caecal microflora.

Key words: broilers, phytogenic additive, performance, jejunal morphology, caecal microflora

Introduction

Modern strategies in broiler production are there to improve profitability, addressing productivity, legislation and consumers' requirements. A ban on the use of antibiotics as growth promoters in 2006 (*Castanon, 2007*) has led to a need for finding efficient yet safe additives for improving production performances with no negative effects on animal health and welfare, quality of food of animal origin, human health and the environment (*European Commission, 2003*). As replacement for antibiotics, most frequently used alternative growth stimulators in broiler production are probiotics, prebiotics, enzymes, acidifiers, antioxidants and phytogenic additives (*Perić et al., 2009*). Phytogenic additives, as natural substances, have been recognized as a very promising alternative solution, as they meet the requirements of consumers in terms of food safety and solve the problem of bacteria resistance that occurs as a result of using antibiotics as growth promoters (*Silva Cardoso et al., 2012*).

Phytogenic additives comprise a wide range of plants and spices, as well as their derivatives that, as supplemental to basal broiler diets, positively affect production performances, animal health and quality of products (*Windisch et al., 2008*). Effects of phytogenic additives are mostly related to antimicrobial, antiviral, and antioxidative activities (*B□lükbaşi and Erhan, 2007*).

Some research on broilers indicate that phytogenics have positive effects on production performances (*Hashemi and Davoodi, 2010*; *Steiner et al., 2008*). Positive effects on production performances can be related to effects that phytogenic additives have on caecal microflora (*Roofchaee et al., 2011*; *Giannenas et al., 2003*), digestive enzyme activity (*Basmacioğlu Malayoğlu et al., 2010*) and caecal morphohistology (*Perić et al., 2010*). Positive effects of phytogenic additives on slaughtering performances of broilers were indicated in the research of *Jafari et al. (2011)* and *Mansoub* (*2011).* However, available literature shows that the use of phytogenic additives does not necessarily result in positive effects on production (*Kirkpinar et al., 2011*) and slaughtering performances (*Scheuermann et al., 2009*), nor caecal microflora (*Cross et al., 2007*). The major reasons for different effects of phytogenic additives, according to *Yang et al. (2009),* are differences among plant species that are used as additives, time of their picking, technology of additive production and the interaction between additives and other dietary components. The mechanism of phytogenic additives has not been explained entirely, and *Cross et al. (2007)* point out that the efficiency of phytogenic additives depends on differences in their chemical composition.

The objective of this research is to investigate effects of phytogenic additives as a broiler feed supplement on production and slaughtering performances, morphological parameters and microbiological status of caecum.

Material and Methods

The investigation on the effects of phytogenic additives was conducted through two trials. The first trial covered the period from the day 1 to the day 35, and the second trial from the day 1 to the day 42. Two feeding treatments were studied: a control diet with no supplemental feed additives (K) and a diet with a phytogenic additive supplemented to the control diet (1000g/t of Biomin PEP 1000, during the whole period). Phytogenic additive Biomin PEP 1000 (Biomin® GmbH Austria) comprises essential oregano, anise and citrus oil, as well as fructooligosaccharides. The main composition of diets was the same in both trials and it was in accordance with hybrid requirements.

In the first trial, 304 chickens were studied, in two treatments with four repetitions per pen with 38 chickens. Control weighing of all chickens was done with a precision balance on the day one and in each stage of the trial. The second trial was conducted in two objects, on 16800 chickens, i.e. 8400 chickens per feeding treatment (object). Control weighing of chickens was done with a precision balance, on a 4% sample.

Chickens were fed *ad libitum*. Feed conversion rate was calculated for different stages and for the whole trial, based on data on feed consumption and chicken growth per different periods, whereas all dead chickens were weighed and taken into the calculation. The death of chickens was recorded on a daily basis.

In order to study slaughtering performances, twelve 35-day old chickens and twelve 42-day old chickens of each gender were randomly chosen for the first and second trial respectively, for each feeding treatment. The chickens that were studied for slaughtering performances were processed according to the *Rulebook on quality of poultry meat (1981)*. When calculating the portion of certain carcass parts, they were calculated in relation to "ready to grill" carcass mass.

At the end of the starter period (21 days) and the end of the trial (42 days), 8 broilers from each group were slaughtered and small intestines were removed. Samples of jejunum were fixed in Bouin Solution, and after histological procedure stained with haematoxylin and eosin. Villous height, crypt depth and villous height: crypt depth ratio was determined using the light microscope and software for image analysis (IM1000, Leica Microsystems GmbH, Germany). A minimum of 15 measurements were made for each parameter per chicken.

In the first trial, on the day 21 and 35, and in the second trial, on the day 35 and 42, target types of bacteria were determined with conventional microbiological techniques on 6 samples. 1 g of caecal content of each group was transferred under aseptic conditions into 9 ml of buffered peptone water. Subsequently, it was homogenized by using a Vortex for 60 sec. Following homogenization, tenfold

serial dilution for each sample was made in sterile peptone–salt water until they were diluted to 10^{-8}. The results were expressed as LOG $_{10}$/g caecal content.

For data processing, STATISTICA 7 computer program, ANOVA, MANOVA and LSD post-hoc test were used.

Results and Discussion

The Table 1 shows production performances by feeding treatments in the first and the second trial. In the first trial, the chickens fed with the supplemental phytogenic additive had significantly more favourable feed conversion rate, while the differences in body mass after the starter period and in the whole trial, as well as mortality rate were not statistically significant. In the second trial, the chickens fed with diets with additives had bigger final body mass, more favourable feed conversion rate and lower mortality rate compared to the control.

The positive effect of phytogenic additives on broiler body mass can be linked with the results of the research of *Mountzouris et al. (2011)* and *Perić et al. (2010),* in which phytogenic additives based on oregano, anise and citrus expressed positive effects in terms of increasing chicken body mass.

Table 1 Effect of phytogenic additives on productive performances of broilers

Treatment	Trial 1					Trial 2		
	Body mass, g		Feed conversion		Mortality, %	Body mass, g	Feed conversion	Mort-ality, %
	Day 21	Day 35	1-21 days	1-35 days	1-35 days	Day 42	1-42 days	1-42 days
Control	803.86	1761.20	1.65 [a]	1.74[a]	2.00	2410.00	1.770	5.02
Sd	*4.81*	*50.21*	*0.04*	*0.03*	*2.09*			
Phytogenic	777.46	1753.00	1.52 [b]	1.68[b]	1.88	2471.00	1.725	4.52
Sd	*34.69*	*76.46*	*0.05*	*0.03*	*1.25*			

a-b Values without the same superscript in a column are significantly different (P <0.05)

The determined improvement in feed conversion in both trials is in compliance with the results of *Mansoub (2011)* and *Mountzouris et al. (2011).* The positive effect of phytogenic additives on feed conversion rate could be linked with effects that additives in this research had on caecal microflora, in terms of reducing the number of bacteria (Table 4) and caecal morpho-histology, i.e. elongation of villous height and crypt depth (Table 3).

The Table 2 shows slaughtering performances of broilers, i.e. yield and the portion of breast, thigh and drumstick, and abdominal fat, by feeding treatments.

Table 2 Effect of phytogenic additives on slaughtering performances of broilers

Treatment	„Ready to grill“ carcass	Breast		Thigh and drumstick		Abdominal fat ,g	
		Yield, g	Portion, %	Yield, g	Portion, %	Yield, g	Portion, %
Trial 1							
Control	1216.30	431.23	35.48	350.11	28.79	8.12	0.66
Sd	*42.97*	*17.34*	*1.57*	*21.41*	*1.52*	*4.84*	*0.38*
Phytogenic	1174.05	411.98	35.09	334.12	28.44	10.22	0.87
Sd	*49.45*	*29.83*	*2.06*	*28.03*	*1.72*	*4.10*	*0.35*
Trial 2							
Control	1752	653.00	37.25	459.90	26.24	14.60	0.83
Sd	*61.80*	*43.00*	*1.60*	*22.50*	*0.9*	*10.60*	*0.60*
Phytogenic	1764	648.60	36.76	486.00	27.54	14.40	0.82
Sd	*51.80*	*26.90*	*1.30*	*21.60*	*0.7*	*6.7*	*0.40*

a-b Values without the same superscript in a column are significantly different (P <0.05)

The values obtained for breast portion and thigh and drumstick portion were very approximate, and the differences between them were significant. It can be said that no significant effects of phytogenic additives on the investigated properties were determined, and there were no particular tendencies as a result of feeding treatments. The obtained results are in compliance with the research of *Jia – Chi et al. (2012),* in which no effects on abdominal fat were determined. However, unlike this research, covering the same issue, *Mansoub* (2011) and *Jafari et al.* (2011) determined the biggest breast portion and the lowest abdominal fat in chickens fed with a supplemental phytogenic additive.

The Table 3 shows effects of phytogenic additives on jejunum morphology parameters, by feeding treatments.

Table 3 Effect of phytogenic feed additives on jejunum morphology of broilers

Treatment	Villous height, µm	Crypt depth, µm	Villous/Crypt ratio	Villous height, µm	Crypt depth, µm	Villous/Crypt ratio
	21-day period			42-day period		
Control	923.83	256.95	3.64	1254.06	303.09	4.14
Phytogenic additive	1050.65	267.11	3.95	1175.13	286.12	4.09

a-b Values without the same superscript in a column are significantly different (P <0.05)

Adding supplemental phytogenic additives did not have significant effect on an increase in villous height, crypt depth and their interrelation. The observed

morphological parameters were higher in 21-day old chickens fed with the supplemental phytogenic additive, yet this trend was not determined in 42-day old chickens. The results are in compliance with the research of *Jia – Chi et al. (2012),* who also did not determine the effect of oregano, anise and citrus based supplemental phytogenic additives on villous height, crypt depth and their interrelation in the jejunum. Unlike this research, in the research of *Perić et al. (2010),* adding phytogenic additives on the day 42 resulted in a significant increase in villous height and crypt depth.

Number of bacteria in caecal content in the first and second trial is shown in the Table 4 by feeding treatments and trial periods.

Table 4 Number of bacteria in caecal content (LOG $_{10}$/g caecal content)

Bacteria	Trial 1				Trial 2			
	Control		Phytogenic		Control		Phytogenic	
	Day 21	Day 35	Day 21	Day 35	Day 35	Day 42	Day 35	Day 42
Total aerobic bacteria	9.14	8.48	8.95	8.33	7.77	7.87[a]	7.59	7.39[b]
Echerichiae coli	8.88	7.73	7.85	6.38	7.66	7.03	6.80	6.49
Enterobacteriaceae	9.04[a]	8.02[a]	8.66[b]	6.62[b]	7.60	7.41[a]	6.98	6.17[b]
Enterococcus	6.99	6.02	6.83	5.49	6.07	5.44[a]	5.42	4.52[b]
Spaphylococcus	5.73	4.77[a]	5.75	4.13[b]	5.61	4.36	5.34	4.17
Lactobacillus spp	-	-	-	-	9.00	9.04	8.67	8.69
Clostridiuum	-	-	-	-	7.05	7.01	6.89	6.90

a-b Values without the same superscript in a row are significantly different (P <0.05)

Having analysed data from the Table 4, one can conclude that in both trials and in all periods of time, chickens fed with the supplemental phytogenic additive had lower values of all bacterial target groups. In the first trial, those differences were statistically significant for *Enterobacteriaceae* and *Spaphylococcus*, and in the second trial for *Enterobacteriaceae* and *Enterococcus*. Adding phytogenic additives resulted in the reduction of *Lactobacillus* spp, which is in compliance with the research of *Jia – Chi et al. (2012)*. However, *Mountzoris et al. (2011)* determined a growing tendency of *Lactobacillus* spp after adding phytogenic additives, what is not in compliance with this research, but they obtained similar results regarding the reduction of total bacteria and Clostridium. The reduction in number of *Echerichiae coli* is in compliance with the research of *Roofchaee et al. (2011)*.

Conclusion

Adding phytogenic additives involved giving oregano, anise and citrus based phytogenic additives to chickens of Ross 308 provenance, for improving production performances, intestinal morphology and microbiological status.

Adding phytogenic additives resulted in the improvement of broiler production performances, above all feed conversion rate. A positive effect was achieved on jejunum morphology parameters and caecal microflora. These effects show that phytogenic additives can contribute to the improvement of production performance, and therefore production efficiency as well.

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Uticaj fitogenih aditiva na performanse, morfologiju i cekalnu mikrofloru brojlerskih pilića

M. Vukić-Vranješ, N. Tolimir, Đ. Vukmirović, R. Čolović, V. Stanaćev, P. Ikonić, S. Pavkov

Rezime

Cilj istraživanja je da se ispita uticaj fitogenog aditiva kao dodatka u ishrani brojlera, na proizvodne i klanične performanse, morfološke parametre i cekalnu mikrofloru tovnih pilića provenijence Ross 308. Ispitivanje je zasnovano na dva eksperimentalna ogleda. U oba ogleda ispitivana su dva tretmana ishrane: kontrolna hrana bez aditiva (K) i hrana za dodatkom fitogenog aditiva u osnovnu smešu (1000g/t Biomin PEP 1000, tokom celog oglednog perioda). U prvom ogledu ispitano je ukupno 304 pilića, prema dizajnu 2 tretmana sa 4 ponavljanja po boksu sa 38 pilića. U drugom ogledu ispitivanje je obuhvatilo 16800 pilića, a izvedeno po principu makroogleda, odnosno objekat od 8400 pilića bio je jedinica posmatranja. Rezultati su pokazali da su pilići hranjeni sa dodatkom fitogenog aditiva ostvarili signifikantno povoljniju konverziju hrane. Razlike u telesnoj masi i mortalitetu ogledne i kontrolne grupe nisu bile na nivou statističke značajnosti. Fitogeni aditivi nisu imali signifikantan uticaj na udeo grudi i abdominalne masti. Dodatak fitogenih aditiva nije rezultirao signifikantnim uticajem na morfološke parametre, ali je postojala tendencija povećanja visine resica i dubine kripti. U oba ogleda, grupe pilića sa dodatkom fitogenog aditiva imale su manji broj svih ispitivanih ciljnih bakterijskih grupa, a razlike su bile i na nivou statističke značajnosti za Enterobacteriaceae i Spaphylococcus u prvom i za Enterobacteriaceae i Enterococcus u drugom ogledu. Na osnovu dobijenih rezultata može se zaključiti da je ishrana sa dodatkom fitogenog aditiva indukovala pozitivan uticaj na performanse brojlera, morfološke parametre jejunuma i mikrofloru cekuma.

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