Effect of Nutrition with Propolis and Bee Pollen Supplements on Bacteria Colonization Pattern in Gastrointestinal Tract of Broiler Chickens

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Abstract

For replace of banned antibiotics used as growth promoters in broiler chicken nutrition, the supplements as probiotics, prebiotics, antioxidants, acidifiers and enzymes, has been mainly studied. These alternative substances improved the feed conversion, performance, growth and health of broiler chickens through a mechanisms associated with gastrointestinal tract and bacteria colonization. Propolis and bee pollen belong to the group of naturally occurring substances of animal and plant origin with antioxidant and antimicrobial activity. The scope of this study was to find a counts of *Enterococcus* sp., *Enterobacteriaceae* family and lactic acid bacteria in crop, ileum and caecum of broiler chickens after propolis and bee pollen addition in their nutrition. Lower dosages of propolis and higher dosages of bee pollen in chicken nutrition significantly ($P<0.05$) reduced the count of *Enterobacteriaceae* family isolates in chicken's crops, while the number of beneficial lactic acid bacteria in chicken's crops with presence of propolis was increased. Ileum and caecum of broiler chickens in experimental group of chickens with higher amount of bee pollen had the lowest ($P>0.05$) number of *Enterobacteriaceae* family isolates. The bacteria colonization pattern in gastrointestinal tract of broiler chickens can be affected also by combination of nutrition supplements and pH value which can provide the advantage to some bacteria and influenced their competitiveness.

Keywords: bee pollen, broiler chickens, gastrointestinal tract, gut microflora, propolis

1. Introduction

Subsequent to banning of use of antibiotics as growth promoter sin poultry nutrition, numerous studies turned to finding of alternative solutions, i.e. other, natural substances, which would have positive effect on chicken growth and feed conversion [1]. The most often additives evaluated in poultry nutrition were probiotics, prebiotics, antioxidants, acidifiers and enzymes.

Gut microflora has significant effects on host nutrition, health, and growth performance [2] by interacting with nutrient utilization and the development of gut system of the host. This interaction is very complex and, depending on the can have either positive or negative effects on the health and growth of the chickens. For example, when pathogens attach to the mucosa, gut integrity and function are severely affected and immune system threatened [3, 4]. Gut microflora is a nutritional "burden" in fast-growing broiler chickens [5, 6] since an active microflora component may have an increased energy requirement for maintenance and a reduced efficiency of nutrient utilization. The focus of alternative strategies has been to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance are improved [7]. One alternative for the improving of the broiler chicken health and performance may be

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incorporation of propolis and bee pollen into broiler diets. Propolis and bee pollen belong to the group of naturally occurring substances of animal and plant origin with antioxidant and antimicrobial activity. Bioactive compounds of propolis and bee pollen (flavonoids, phenolic acids and their derivatives) are responsible for its bactericidal, antiviral antifungal, antioxidant effect [8-10].

The colonization pattern of the gastrointestinal tract of broiler chickens by microorganisms might be affected by type of diet [11]. The objective of this study was to determine the effect of both feed supplements propolis and bee pollen to colonization pattern of the gastrointestinal tract of broiler chickens.

2. Materials and methods

Breeding conditions:

The experiment included 300 one day-old chickens, which were divided into five groups: control and four experimental groups. The tested chickens of hybrid combination Ross308 were fed by commercial feed mixtures HYD 01 (until to 21st day of their age) and HYD 02 (from 22nd to 42nd day of their age) (Table 1).

Table 1. Ingredients and nutrient composition of experimental diets

Ingredient (%)	Starter HYD-01 (1 to 21 days of age)	Growther HYD-02 (22 to 42 days of age)
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48% N)	21.30	18.70
Fish meal (71% N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysin	0.05	0.07
Methionin	0.15	0.22
Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 05 %	0.50	0.50
Nutrient composition (g/kg)		
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
Linoleic acid	13.51	14.19
ME_N (MJ.kg^{-1}), calculated	12.02	12.03

The first and second experimental broiler chicken group was fattening by a feed mixture with propolis extract supplement in dose 400 (P1) respectively 800 mg. kg^{-1} (P2). The third and fourth experimental broiler chicken groups was fattening by a feed mixture with bee pollen in dose 15 g. kg^{-1} (P3) respectively 45 g. kg^{-1} (P4). Broiler chickens were fed by ad libidum system and the fattening period was during 42 days. The broiler chickens were breeding in a cage conditions. Each cage was equipped with feed disperser and water intake was ensured ad libitum through a self feed-pump.

Sampling:

After fattening, broilers were processed by stunning, bleeding, scalding, and picking. For microbial examination (30 samples) were whole crops, ileum and caecum aseptically removed from five carcasses per group (10 from control group) and were placed in separate sterile plastic bags. The chyme of each parts of gastrointestinal tract was weighed and properly homogenized in

ten-fold amount of saline. Serial dilutions were made also in saline and plated on an appropriate agar medium.

Microbiological analysis

The chyme samples for enumeration of total lactic acid bacteria count were cultured on diagnostic MRS agar (HiMedia, India). Samples were incubated at temperature 37±1°C for five days under anaerobic conditions. The chyme samples for enumeration of family *Enterobacteriaceae* isolates were cultured on selective diagnostic VRBG agar (HiMedia, India) at temperature 37±1°C for 24±2 hours. The counts of enterococci were determined on selective diagnostic Slanetz–Bartley agar (Biokar Diagnostic, Pantin, France) at temperature 37±1°C for 48±2 hours.
The pH value of the chickens crops, ileum and caecum contents were measured electronically by pH apparatus Gryf 209L.

3. Results and discussion

In accordance with the findings of the study, the populations of lactic acid bacteria, *Enterobacteriaceae* and *Enterococcus* sp. in crop, ileum and caecum are shown in Table 2 and Figure 1.

Table 2. Occurrence of microorganisms and pH value in different parts of broiler chickens gastrointestinal tract after various doses of bee pollen and propolis addition

	C	P1	P2	P3	P4
Crop [log cfu. g^{-1}]					
Lactic acid bacteria	7.87	8.49	7.90	7.16	6.94
Enterobacte-riaceae	7.59	5.72	6.58	5.66	4.60
Enterococcus sp.	4.81	≤ 2	≤ 2	5.09	3.39
pH	4.77	4.57	4.60	4.40	4.05
Ileum [log cfu. g^{-1}]					
Lactic acid bacteria	7.46	7.45	6.30	8.00	7.79
Enterobacte-riaceae	5.90	7.49	5.93	6.94	5.68
Enterococcus sp.	3.76	≤ 2	≤ 2	6.54	4.41
pH	6.31	6.09	6.54	7.08	6.86
Caecum [log cfu. g^{-1}]					
Lactic acid bacteria	8.67	8.36	7.96	8.08	5.95
Enterobacte-riaceae	8.20	8.07	7.95	8.15	7.74
Enterococcus sp.	6.81	≤ 2	≤ 2	7.48	5.40
pH	6.63	6.74	6.88	6.91	6.64

C=control group, P1=propolis in dose 400 $mg.kg^{-1}$, P2=propolis in dose 800 $mg.kg^{-1}$, P3=bee pollen extract supplement in dose 15 $g.kg^{-1}$, P4=bee pollen extract supplement in dose 45 $g.kg^{-1}$

It must be note that broiler chickens were not subjected to feed withdrawal. According to Hinton et al. (2000) [12] the absence of feed, resulted in a decrease in the population of lactic acid bacteria and an increase in the pH of the crop and then extended feed withdrawal times may increase the possibility that crop of broilers may contain food-borne pathogens.
Due to antimicrobial effect of propolis the *Enterococcus* sp. was inhibited throughout whole gastrointestinal tract of broiler chickens in compare with other groups. Higher dosages of bee pollen (P4) and lower dosages of propolis (P1) in chicken nutrition significantly ($P<0.05$) reduced count of *Enterobacteriaceae* family isolates in chicken's crops, while the number of beneficial lactic acid bacteria in chicken's crops with presence of propolis was increased. The pH value was the lowest in crops of P4 experimental group where was at the same time observed the highest decline of isolates but also the highest decline of lactic acid bacteria was observed. According to Basin et al. (2006), Kumova et al. (2002) and Bankova, et al. (2007) [13-15] the decline of *Enterobacteriaceae* family counts in chicken's crops may be associated with antibacterial activity of bee pollen and propolis.

Ileum and caecum of broiler chickens in P4 experimental group had the lowest (P>0.05) number of *Enterobacteriaceae* family isolates than control and others experimental groups. However the count of *Enterococcus* sp. isolates and pH

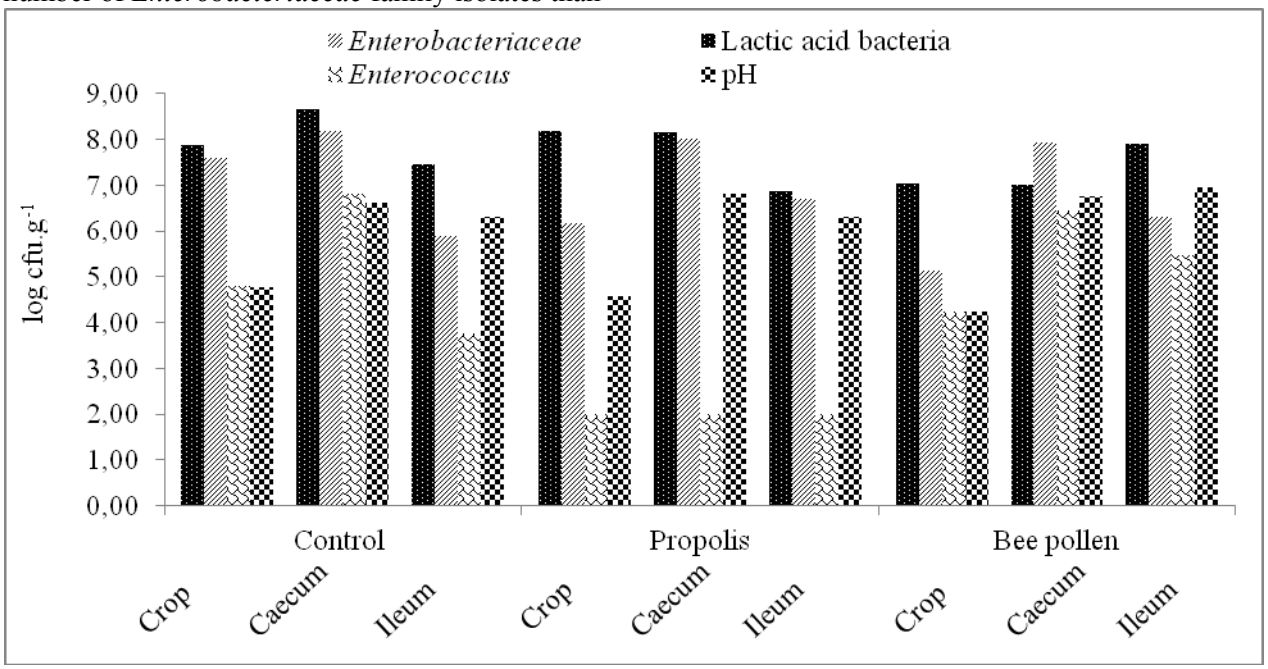

Figure 1. Occurrence of microorganisms and pH value in various parts of broiler chickens gastrointestinal tract after propolis and bee pollen addition without dosage dividing
*counts of *Enterococcus* sp. in crop and caecum after propolis addition were lower than 2 log cfu. g^{-1}

value significantly increase (P<0.05) in ileum of broiler chickens with nutrition enriched by bee pollen (P3, P4). Tekeli et al. (2010) [16] found comparable count of lactic acid bacteria (6.55 log cfu.g^{-1}) in jejunum (part above ileum) of broiler chickens after propolis addition. In comparison with our results, Rahmani and Speer (2005) [17] found similar values of pH in ileum of broiler chickens after natural supplement addition in their diet. The higher increase of lactic acid bacteria count in ileum of P3 chicken's group was not significant. Also Vidanarachchi *et al*. (2006) [18] revealed that plant extract additives increased the numbers of lactic acid bacteria in the ileum and cecum of broilers while significantly reducing the numbers of total anaerobic, coliform and *C. perfringes* bacteria. Alp et al. (1999) [19] reported that inclusion of antibiotics and an organic acid mixtures containing lactic, fumaric, propionic, citric and formic acids separately or in combination reduced the *Enterobacteriacae* count in the ileum of broilers.

Caecum of broiler chickens without nutrition with propolis and bee pollen had not significantly higher count of any observed bacteria (except counts of enterococci in P3 group). The differences among pH values were not significant ($P < 0.05$). Ramarao et al. (2004) [20] observed that the total bacterial, coliform, and *E. coli* counts in crop and caecal contents were low in broilers fed gut acidifier and opined that gut acidifier can safely replace antibacterial compounds in broiler chicken diets with beneficial effects on the intestinal bacterial colonization and resistance to *E. coli* challenge.

4. Conclusions

It was found that occurrence of both beneficial and pathogen microoragnisms in gastrointestinal tract of broiler chickens was affected by nutrition with propolis and bee pollen supplement. The bacteria colonization pattern in gastrointestinal tract of broiler chickens can be affected also by combination of nutrition supplements and pH value which can provide the advantage to some bacteria and influenced their competitiveness.

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