

Effect of Chinese Propolis Supplementation on Ross Broiler Chicks: Microbial Population in Fecal matter and Litter

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Abstract

Propolis has been considered a good candidate for health amelioration and disease prevention over a long period of time. Effect of feed supplementation with Chinese ether extract propolis was evaluated for the antibacterial action on (Ross 308) broiler chicks and their litter over 35 days. The addition of different levels of propolis (100, 250 and 750 mg/kg diet) on total aerobic and coliform bacteria as well as *Lactobacillus spp.* and bifidobacteria were determined. Supplementation of propolis significantly increased ($p < 0.05$) the desired stimulating effect of *Lactobacillus spp.* and Bifidobacteria in fecal samples of all treatment groups compared to control group. However, propolis did not have an impact to reduce the total aerobic and coliform bacteria compared to control group in both fecal and litter samples. In conclusion, Chinese propolis improved the beneficial normal gut microflora and had a limited effect on the total aerobic and coliform bacteria in broiler chicks and litter quality.

Keywords: Propolis; Bacterial count; Gut microflora; Litter; Broiler.

Introduction

For restriction of antibiotic used as a growth promoter in broiler chicken, the supplements of natural products as probiotics, prebiotics, organic acids, antioxidants, enzymes and plant extracts has been studied as a candidate replacement (Capcarova *et al.*, 2010). These alternative substances improved the feed conversion, performance, growth and health of broiler chickens through a mechanisms associated with gastrointestinal tract and bacteria colonization (Kročko *et al.*, 2012).

Propolis is a natural glue-like substance produced by bees from plant seedlings and buds. It is obtained as a result of the biochemical alteration of the resinous materials and plant excretion by the enzymes secreted from the glands of bees. Propolis proved to have numerous biological activities including antioxidant, anti-microbial, anti-carcino-

genic, anti-fungal, anti-viral, anti-ulcer, immunostimulatory and anti-inflammatory properties. (Atungulu *et al.*, 2007 and Barros *et al.*, 2007). The antibacterial activity of propolis is contributed to flavonoids, phenolic acids and their derivatives (Tosi *et al.*, 2007; Erkmen and Özcan, 2008). The antibacterial mechanism was achieved through suppressing bacteria cell development by blocking their cell mitosis and protein synthesis. Another mechanism is through direct destructing bacteria cells by disarranging their cell cytoplasm, cell membranes, and also their cell walls (Takaisi-Kikuni and Schilcher, 1994).

Poultry are very sensitive to pathogenic bacteria such as *E. coli*, *Salmonella spp.*, *Clostridium perfringens*. Some bioactive substances derived from higher plants have been reported to kill pathogenic bacteria species while increasing beneficial bacteria such as lactic acid and bifidobacteria (Gunal *et al.*, 2006). The stimulation of such beneficial bacteria results in an effective protection against pathogenic microorganisms and a balanced intestinal

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microflora (Vidanarachchi *et al.*, 2006). Intestinal microflora provide natural barrier against harmful bacteria that enter the intestine; they inhibit growth of exogenous and pathogenic bacteria, and produce bacteriocins or other substances thus enhancing the immune system (Tannock, 1988; Barrow, 1992; Gibson and Roberfroid, 1995; Gong *et al.*, 2002; Van der Wielen *et al.*, 2002; Lan *et al.*, 2005). The competition between intestinal microflora and exogenous or pathogenic organisms for limited carbon sources, the presence of antibacterial compounds, and production of volatile fatty acids can control the growth and translocation of the exogenous or pathogenic organisms (Barnes, 1977). A well-established normal intestinal flora compete with pathogens and hence decrease the risk of salmonellosis, *Clostridium perfringens*-associated lesions, campylobacteriosis, or colibacillosis (Weinack *et al.*, 1981; Soerjadi *et al.*, 1982; Fukata *et al.*, 1991; Barnes, 1997; Craven *et al.*, 1999; Kaldhusdal *et al.*, 2001) and/or protect against colonization of the intestine by pathogens (Mead, 2000).

In poultry production, litter is a potential reservoir and transmission vehicle for pathogens and potential pathogens (Montrose *et al.*, 1985, Weinack *et al.*, 1985, Willis *et al.*, 2002, Lu *et al.*, 2003). Intensive systems generate large quantities of litter that has been traditionally applied to agricultural soils for decades as an organic fertilizer (Moore *et al.*, 1995). In addition, animal and poultry manure is extensively used as a cheaper alternative to artificial fertilizer to increase phytoplankton production (natural fish food) in fish ponds from different countries (Knud-Hansen, 1993; Little and Edwards, 1999; Petersen *et al.*, 2002; EAHMI, 2008). The importance of enhancing a safe recycling of poultry litter and its nutrients increased opportunities to market their energy and nutrients to agricultural and non-agricultural uses. Based on the work of several authors propolis may have a beneficial effect on the gastrointestinal microflora of poultry (Nováková *et al.*, 2008; Kačániová *et al.*, 2011). However, its antimicrobial activity depends on the physical structure, concentration and the dose intensity (Dias *et al.*, 2012). In addition, there is no information about the antimicrobiological effects of propolis on broilers litter. Therefore, the objective of the present study was to determine the effect of ether extract propolis (EEP) supplementation on intestinal and litter mi-

crobiology in broiler chicks. It was expected that EEP could be efficient for replacing the wide therapeutic antibiotics and able to reduce cost of medication in broilers due to the antibacterial properties of these extracts.

Materials and methods

Birds and treatments

Sixty four, one day old broiler chicks (Ross 308) were divided into four groups of 16 birds each in a completely randomized design. The experiment was planned in accordance with animal welfare. Feed and water were offered to allow for ad libitum consumption. The groups were fed as follow: 1- Basal diet, no additives (Control), 2- Basal diet plus 100 mg propolis /kg diet, 3- Basal diet plus 250 mg propolis /kg diet, 4- Basal diet plus 750 mg propolis /kg diet. The addition of propolis to the diet was started at day 3. The ingredients of the diet and calculated energy uptake/ Kg diet were presented in Table 1. Birds were given a starter diet to 21 days of age followed by a grower diet till the end of the experiment. The experiment was conducted in an environmentally controlled floor house of clean softwood shavings as litter material. Bird density was 20 kg per square meter. The lighting cycle was maintained at 23 hours/day. The ambient temperature in the experimental house was maintained at 34°C during the first week and gradually decreased by 3 °C during the second and third weeks, and was fixed at 26 °C thereafter. Average relative humidity ranged between 40 to 60 %. Ventilation was provided by negative pressure with fans. Chicks were vaccinated via their drinking water against New Castle Disease at days 6, 14, 21, and 32 and against Infectious Bursal Disease at days 10, 18 and 25. Ether extracted propolis was purchased from Dalian Tianshan Industrial Co.TM, Ltd. Changjiang Road, Dalian, Liaoning, China.

Sampling and microbiological analyses

Eight Faecal samples and three pooled litter samples were collected from each group at day 8, 18 and 35 for microbiological analyses. Fresh fecal samples from birds were aseptically collected on a sterile sheet and immediately transferred to sterile plastic bags. Litter samples were collected from each group using sterile plastic bags. Samples were

Table 1. Composition of the experimental diets (g/kg) and energy uptake (Kg/diet)

Ingredients	Starter	Grower
Com	50.5	60.05
Fish meal	3.5	3
SBM	36.75	29
Sunflower oil	6	4.7
Dicalcium phosphate	1.5	1.5
Ground lime stone	1	1
Salt	0.3	0.3
Lysine	0.1	0.1
Methionine	0.1	0.1
Premix	0.25	0.25
Calculated energy uptake/ Kg diet		
E (Kcal/kg)	3202.465	3208.048
CP (%)	22.977	20.0363

placed on ice and transported to the laboratory in an ice box for bacteriological analysis that was carried out within 2 hours. Bacteriological analyses were performed with a total of 96 fecal and 36 litter samples. Fresh fecal microbial population were accessed for the total viable aerobic counts, coliform, *Lactobacillus spp.* and Bifidobacterium by using culture techniques described by Hu *et al.* (2012). Fresh fecal samples (1g) were placed into preweighed 15 mL sterile plastic tubes containing 9 ml sterile saline (0.85%) which was called the initial fecal dilution. Litter samples (1 g) were placed into sterile glass bottle containing 99 ml sterile saline. Tenfold dilutions were spread in duplicate onto standard plate count agar (Lab M Limited, Lancashire BL9 6As, UK) and MacConkey agar (Lab M Limited, Lancashire BL9 6As, UK). Standard plate count agar and MacConkey agar plates were incubated aerobically at 37°C for 24 hours for total aerobic bacteria and coliform counts in fecal and litter samples, respectively (Halkman *et al.*, 1994; Merk, 1998). For enumeration of *Lactobacillus spp.* bacteria and bifidobacteria, the initial fecal dilution was tenfold serially diluted in 0.85% sterile saline solution. From each dilution, 1 mL was inoculated on MRS agar (Biolife, Milan, Italy) and MRS agar supplemented with L-cystiene hydrochloride for *Lactobacillus spp.* and bifidobacteria, respectively. Lactobacilli and Bifidobacteria were anaerobically incubated (SHEL LAB CO₂ Incubator, SHELDON manufacturing, Inc, USA, Model no. 2406-2) at 37°C for 48 hours, counted and recorded as colony forming units per gram of fecal material. All microbiological analyses were performed in duplicate and the average values expressed as log₁₀ cfu g⁻¹ fecal and used for statisti-

cal analysis. *Lactobacillus spp.* and Bifidobacteria were counted in faecal samples collected at days 18 and 35 using MRS Agar (pH 5.4,) and MRS Agar supplemented with 0.25% L-cysteine hydrochloride, respectively. Incubation was performed under anaerobic condition at 37°C for 48-72 hours (Asperger and Saad, 1999; Zinedine and Faid, 2007).

Statistical analysis:

Statistical Analysis of data was carried out using SPSS version 11 statistical package programs. A one- way analysis of variance (ANOVA) was performed. Differences in mean values were accepted as being statistically significant if $P < 0.05$. When the effect was significant ($p < 0.05$), means were separated using Tukey's test. The statistical significance was determined at both $P \leq 0.01$ and $P \leq 0.05$ levels.

Results

The results of the application of EEP (100, 250 and 750 mg /kg diet) influenced *Lactobacillus spp.* and Bifidobacteria number in fecal samples at 18 and 35 days are shown in Fig. 1 (a & b).

Lactobacillus spp. and bifidobacterium, in all treatment groups at day 18, have significantly increased with respect to control group. At day 35, only counts of *Lactobacillus spp.* were significantly higher comparing with control group ($P = 0.013$). Moreover, non-significant differences were observed in *Lactobacillus spp.* and bifidobacteria counts between groups of different propolis concentrations. The highest *Lactobacillus spp.* count

(9.4 log₁₀ cfu/g and 13.38 log₁₀ cfu/g) was observed in broilers received 750 mg and 250 propolis/kg diet at days 18 and 35, respectively. However, the highest bifidobacteria count (8.75 log₁₀ cfu/g) was reported in the group with 100mg propolis/kg diet at day 18.

Effect of different levels of EEP (100, 250 and 750 mg /kg diet) on fecal samples for total aerobic and coliform counts at different ages (8, 18 and 35 days) of broiler chicks, were presented in Fig. 1 (c & d). The total aerobic count in the control group has a tendency to increase with bird age from day 18 to 35. The same pattern was observed in all treated propolis groups regardless its concentration in the diet. Similar observation in both control and treated propolis groups with the total coliform count in broilers fecal samples which was increased with increasing the age of birds from 8 to 35 days. Propolis administration has no significant impact on the total aerobic and coliform counts in broiler,

except at concentration 100mg/kg diet where a significant increase in total aerobic and coliform at day 35 was observed (Fig. 1 c & d).

The data of total aerobic and coliform count of litter samples collected at days 8, 18 and 35 of the experiment were presented in Fig. 2 (a & b). It can be observed that there was no significant effect of any propolis treatment on the bacterial counts in any litter sample, except, total aerobic count of the treatment group received 100mg propolis/kg feed showed a significantly higher count than the control group at days 8 and 18.

Discussion

The desired effect of propolis on *Lactobacillus spp.* and *Bifidobacterium* could be attributed to the regulating and protecting role of these bacteria on chicks. Gut microflora are nutritional “burden” in fast-growing broiler chickens (Lan et al., 2005;

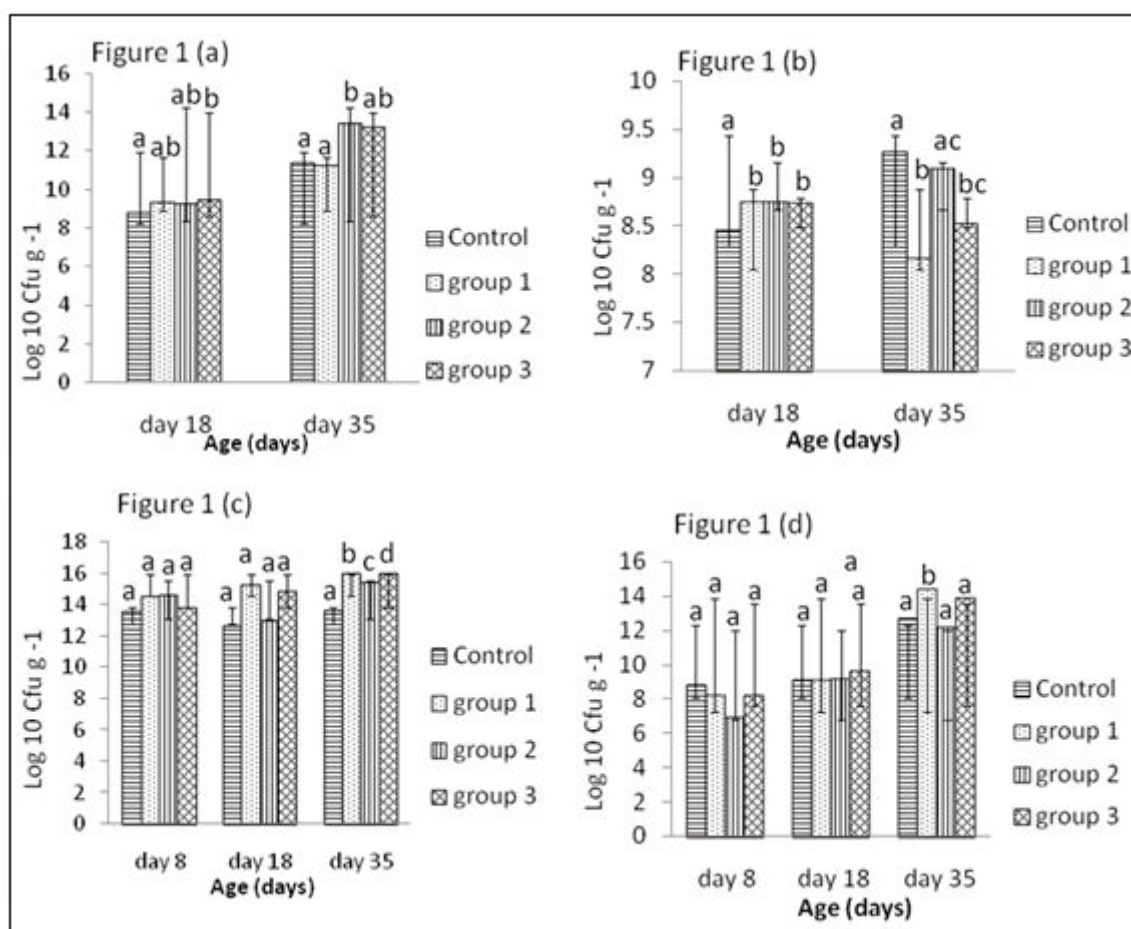


Fig. 1 (a, b, c and d). Log₁₀ cfu of the different types of bacteria in fecal samples of broiler chicken. a&b: Lactic acid and Bifidobacteria number in samples collected at day 18 and 35 of the experiment, respectively. c & d: Total mesophilic and coliform count in samples collected at day 8, 18 and 35 of the experiment, respectively. The groups were as follow: Control (Basal diet), Group 1, Basal diet plus 100 mg propolis /kg diet, Group 2, Basal diet plus 250 mg propolis /kg diet, Group 3, Basal diet plus 750 mg propolis /kg diet. abc: means with different letters are significantly different (p<0.05)

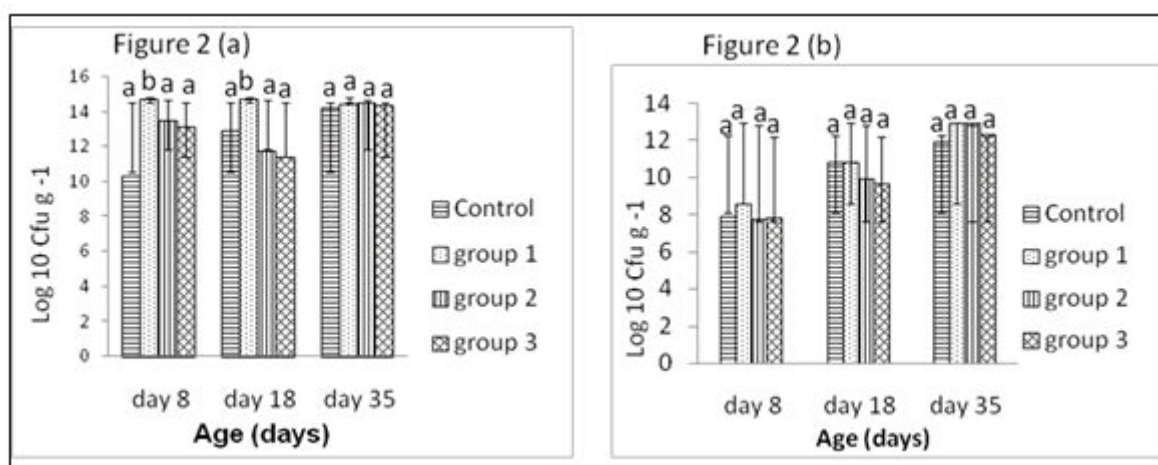


Fig. 2. Log₁₀ cfu of total mesophilic and coliform count of litter samples collected at days 8, 18 and 35 of the experiment as shown in a & b respectively.

Adil *et al.*, 2011), since active microflora component may have an increased energy requirement for maintenance and a reduced efficiency of nutrient utilization (Kročko *et al.*, 2012). The stimulation of such beneficial bacteria generates an effective protection against pathogenic microorganisms and a balanced intestinal microflora (Vidanarachchi *et al.*, 2006). The administration of *B. bifidum* to broiler chickens in drinking water significantly decreased the incidence of cellulitis and the increase of *Lactobacillus spp.* in newborn ducks and chicks preceded the development of weight gain (Estrada *et al.* 2001; Angelakis and Raoult, 2010). The results suggested that propolis level as low as 100 mg/kg diet, for 18 days, could be enough to enhance the growth and multiplication of beneficial gut microflora in broilers. This observation is in agreement with previous reports on the positive effect of propolis on gut microflora. Propolis extracts supplemented in the ration both separately and in combination with *Zingiber officinale* proved to stimulate lactic acid bacteria in broilers (Tekeli *et al.*, 2010). In another report, the number of beneficial lactic acid bacteria in chicken's crops with presence of propolis was increased while the number of this bacteria in chickens's ileum and caecum was decreased in both 400 and 800 mg propolis/kg diet (Kročko *et al.*, 2012). Many reports revealed the positive effect of some plant extracts on the gut microflora of birds. Plant extract additives increased the numbers of lactic acid bacteria (bifidobacteria ve lactobacilli) in the ileum and caecum of broilers (Guo *et al.*, 2004; Vidanarachchi *et al.*, 2006). On the contrary, *Lactobacillus spp.* number has no statistically significant differences in the

trial with chickens after application of propolis (Kačániová *et al.*, 2012).

The lower gastrointestinal tract of most animal species including poultry is normally populated by large number of microorganisms, and through various competitive niches and virulence capabilities, some are able to survive (Kačániová *et al.*, 2012). The effect of propolis was less pronounced, suggesting that the inhibition of total aerobic and coliform bacteria may be dose related. Lower concentration of propolis may contain reduced level of the active biological antibacterial substance/s. The presence of certain bacteriostatic/bactericidal chemicals in the growth medium at a concentration lower than a critical inhibitory level could enhance the growth of an organism that otherwise would have been inhibited/ killed by higher concentrations, a phenomenon known as hormesis (Calabrese and Baldwin, 2003). This situation alerts the importance of dose adjustment of propolis before using in broilers to be above the minimum inhibitory concentration (MIC) values, in order to minimize the risk of encouraging bacterial growth. In accordance with our results, Tekeli *et al.* (2010) recorded a significant increase in total aerobic bacteria after supplementation with of *Zingiber officinale* plant (240 ppm) and propolis (1000 ppm) comparing with the control ($P < 0.05$).

Coliform bacterial population in broilers displayed a reduced sensitivity especially at low propolis concentration. In accordance with other results, gram negative bacterium *E. coli* showed either very low sensitivity or total insensitivity against propolis (Digrak *et al.*, 1995; Marcucci, 1995; Bonvehí and Coll, 2000; Sforcin *et al.*,

2000). The most plausible explanation is their outer membrane that inhibits and/or retards the penetration of propolis at lower concentrations. After a certain threshold concentration, however, the impermeability of this membrane is disturbed resulting in the movement of bioactive components of propolis into the cell interior, resulting in cell inhibition or death. Another explanation may be the resistance of gram negative bacteria to propolis due to the possession of multidrug resistance pumps (MDRs), which extrude amphipathic toxins across the outer bacterial membrane (Tegos *et al.*, 2002). The MDRs could be very effective at lower concentration of propolis and extrude those molecules that crossed the outer membrane barrier. This pumping potential of the MDRs can be saturated at a certain threshold concentration and the rate of penetration and accumulation of antimicrobials in the cell interior increases, resulting in antimicrobial effect at higher concentrations (Lomovskaya and Lewis, 1992; Nikaido, 1999; Zgurskaya and Nikaido, 1999; Lewis and Lomovskaya, 2001). From these obtained results, it can be suggested that higher aerobic count in treated propolis groups may be attributed to the higher coliform counts in the examined fecal samples of poultry. In agreement with our results, Enterobacteriaceae count in ileum and caecum of chicks either remains unchanged or increases when propolis was added in a food mixture at concentrations 400 and 800 mg/kg diet (Kročko *et al.*, 2012). On the contrary, other researchers reported the antibacterial effect of propolis on coliforms. Results of Tekeli *et al.* (2010) revealed that supplementation of Zingiber officinale and propolis extracts alone or in combination significantly decrease ($P < 0.05$) the existence of coliform bacteria in all treatment groups. In addition, propolis significantly and dose dependently controls the microbial load, particularly the *E. coli* and clostridium rates comparing the control group (Rahmani *et al.*, 2006). Plant extract additives significantly reduced the numbers of total anaerobic, coliform and *C. perfringens* bacteria in the ileum and caecum of broilers (Vidanarachchi *et al.*, 2006). High aerobic and coliform bacterial count assumed to be responsible for the risk of developing clinical and subclinical infection as well as impairment of the absorption of nutrients in the intestine and depression of growth.

Our study found no influence of propolis on reducing litter microbiology. Large quantities of

poultry litter, have been produced by intensive poultry production systems, Average litter production rates were 228.2 g of dry litter material per kg of live broiler weight (g/kg) per flock (Coufal *et al.*, 2006). Poultry litter has been traditionally applied to agricultural soils for decades as an organic fertilizer, (Moore *et al.*, 1995). In addition, litter has the potential for being recycled as food nutrient in fish farms (Petersen *et al.*, 2002; EAHMI, 2008). However, because litter contains many essential nutrients, high bacterial loads could result if litter is not prepared at acceptable conditions. Therefore, improving litter microbial quality can help to reduce infection, improve production, performance and lower costs for poultry producers. Poultry litter containing high microbial population is considered of low value and unhygienic problems could result from reuse in plant or animal. A study on the pathogenesis of cellulitis demonstrated that scratching the skin of chicken broilers and then exposing them to litter seeded with *E. coli* cells induced cellulitis in many chickens (Macklin *et al.*, 1999). Litter and other management practices also can change microbial composition of the chicken gut directly by providing a continuous source of bacteria or indirectly by influencing the defense mechanisms of the birds (Apajalahti *et al.*, 2004). Studies with higher doses of propolis could be worthy of future work.

Conclusion

Under the conditions of this study, it can be concluded that *Lactobacillus spp.* and Bifidobacteria are positively influenced by supplementation of propolis. However, the highest propolis level in this experiment could be insufficient to exert an antimicrobial action on the total aerobic and coliform bacteria in broiler chicks. Further investigations are needed for justification the proper type, dose and regimen of propolis, as a natural additive, for efficient commercial application in organic chicken production.

References

- Adil, S., Banday, M.T., Bhat, G.A., Mir, M.S., 2011. Alternative strategies to antibiotic growth promoters. A review in Vet Scan 6, article 76.
- Angelakis, E., Raoult, D., 2010. The Increase of *Lactobacillus Species* in the Gut Flora of Newborn Broiler Chicks and Ducks Is Associated with Weight Gain.

PLOS ONE 5(5), e.0010463.

- Apajalahti, J., Kettunen, A., Graham, H., 2004. Characteristics of the gastrointestinal microbial communities, with special reference to chicken. *World's Poultry Science Journal* 60, 223–232.
- Asperger, H., Saad, N. M., 1999. A comparison study between Oxyrase anaerobic agar plates and conventional anaerobic method for the enumeration of lactic acid and bifidobacteria from fermented milk. *Milchwissenschaft* 54(11), 613–616.
- Atungulu, G., Miura, M., Atungulu, E., Satou, Y., Suzuki, K., 2007. Activity of gaseous phase steam distilled propolis extracts on peroxidation and hydrolysis of rice lipids. *Journal of Food Engineering* 80, 850–858.
- Barnes, E.M., 1977. Ecological concepts of the anaerobic flora in the avian intestine. *American Journal of Clinical Nutrition* 30, 1793–1798.
- Barnes, E.M., 1997. Ecological concepts of the anaerobic flora in the avian intestine. *American Journal of Clinical Nutrition* 30, 1793–1798.
- Barros, M.P., Sousa, J.P., Bastos, B.J.K., Andrade, S.F., 2007. Effect of Brazilian green propolis on experimental gastric ulcers in rats. *Journal of Ethnopharmacology* 110, 567–571.
- Barrow, A.P., 1992. Probiotics in chickens. *The Scientific Basis*. R. Fuller, ed. Chapman & Hall. New York, In *Probiotics* 225–259.
- Bonvehí, J.S., Coll, F.V., 2000. Evaluation of purine alkaloids and diketopiperazines contents in processed cocoa powder. *European Journal of Food Research Technology* 210, 189–195.
- Calabrese, E.J., Baldwin, L.A., 2003. HORMESIS: The Dose-Response Revolution. *Annual Review of Pharmacology and Toxicology* 43, 175–97.
- Capcarova, M., Chmelnicna, L., Kolesarova, A., Massanyi, P., Kovacik, J. 2010. Effect of *Enterococcus faecium* M 74 strain on selected blood and production parameters of laying hens. In *British Poultry Science*, vol. 51 (5), 614–620.
- Coufal, C.D., Chavez, C., Niemeyer, P.R., Carey, J.B., 2006. Measurement of broiler litter production rates and nutrient content using recycled litter. *Poultry Science* 85(3), 398–403.
- Craven, S.E., Stern N.J., Cox N.A., Bailey J.S., Berrang M., 1999. Cecal carriage of *Clostridium perfringens* in broiler chickens given mucosal starter culture. *Avian Disease* 43, 484–490.
- Dias, L.G., Pereira, A.P., Estevinho, L.M., 2012. Comparative study of different Portuguese samples of propolis: Pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. *Food and Chemical Toxicology* 50(12), 4246–4253.
- Digrak, M., Yilmaz, O., Ozcelik, S., 1995. In vitro antimicrobial effect of propolis collected in Elazig region. *Turkish Journal of Biology* 19, 249–257.
- EAHMI, 2008. *Livestock and Poultry Manure Use in Aquaculture in the Philippines*. Philippine Environmental Animal Health management initiative Policy Brief 2, 1–4.
<http://eahmi.org/images/pdfdocs/Livestock%20and%20Poultry%20Manure%20Use%20in%20Aquaculture%20in%20the%20Philippines.pdf>
- Erkmen, O., Özcan, M.M., 2008. Antimicrobial effect of Turkish propolis, pollen, and Laurel on spoilage and pathogenic food-related microorganisms, *Journal of Medicinal Food* 11, 587–592.
- Estrada, A., Wilkie, D.C., Drew, M., 2001. Administration of *Bifidobacterium bifidum* to chicken broilers reduces the number of carcass condemnations for cellulitis at the abattoir. *Journal of Applied Poultry Research* 10, 329–334.
- Fukata, T., Hadate, Y., Baba, E., Arakawa, A., 1991. Influence of bacteria on *Clostridium perfringens* infections in young chickens. *Avian Disease* 35, 224–227.
- Gibson, G.R., Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition* 125, 1401–412.
- Gong, J., Forster, R.J., Yu, H., Chambers, J.R., Wheatcroft, R., Sabour, P.M., Chen, S., 2002. Molecular analysis of bacterial populations in the ileum of broiler chickens and composition with bacteria in the cecum. *FEMS Microbiology Ecology* 41, 171–179.
- Gunal, M., Yayli, G., Kaya, N., Karahan, N., Sulak, O., 2006. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. *International Journal of Poultry Science* 5(2), 149–155.
- Guo, F.C., Williams, B.A., Kwakkel, R.P., Li, H.S., Li, X.P., Luo, J.Y., Li, W.K., Verstegen, M.W.A., 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry Science* 83, 175–182.
- Halkman, K., Dogan, H., Noveir, M.R., 1994. *Salmonella and E. coli* Bacteria in Foodstuffs Comparison of Methods for Determining and be Presumed. *Food Technology Association Publication*, Ankara 21, p. 93.
- Hu, C.H., Gu, L.Y., Luan, Z.S., Song, J., Zhu, K., 2012. Effects of montmorillonite – zinc oxide hybrid on performance diarrhea, intestinal permeability and morphology of weanling pigs. *Animal Feed Science and Technology* 177, 108–115.
- Kačániová, M., Rovná, K., Arpášová, H., Čuboň, J., Hleba, L., Pochop, J., Kunová, S., Haščík, P., 2012. In vitro and In vivo antimicrobial activity of propolis on the microbiota from gastrointestinal tract of chickens, *Journal of Environmental Science and Health, Part A* 47, 1665–1671.
- Kačániová, M., Vukoviá, N., Bobková, A., Fikselová, M., Rovná, K., Haščík, P., Čuboň, J., Hleba, L., Bobko, M., 2011. Antimicrobial and antiradical activity of Slovakian honeydew honey samples. *Journal of Microbiology, Biotechnology and Food Sciences* 1, (3) 354–368.
- Kaldhusdal, M., Schneitz, C., Hofshagen, M., Skjerve, E., 2001. Reduced incidence of *Clostridium perfringens*-associated lesions and improved performance in broiler chickens treated with normal intestinal bacteria from adult fowl. *Avian Diseases* 45, 149–156.
- Knud-Hansen C.F., 1993. The role of chicken manure in the production of Nile tilapia, *Oreochromis niloticus*. *Aquaculture and Fisheries Management* 24, 483–493.

- Kročko, M., Čanigová, M.: Bezeková J., Lavová, M., Haščík, P., Ducková, V., 2012. Effect of Nutrition with Propolis and Bee Pollen Supplements on Bacteria Colonization Pattern in Gastrointestinal Tract of Broiler Chickens. *Animal Science and Biotechnologies* 45 (1) 63-67.
- Lan, Y., Verstegen, M.W.A., Tamminga, S., Williams, B.A., 2005. The role of the commensal gut microbial community in broiler chickens. *World's Poultry Science* 61, 95-104.
- Lewis, K., Lomovskaya, O., 2001. Drug efflux. In *Bacterial resistance to Antimicrobials, Mechanisms, Genetics, Medical Practice and Public Health*. Lewis, K. Salyers, A., Taber, H., Wax, R. (eds). Marcel Dekker, New York pp. 61-90.
- Little, D.C., Edwards, P., 1999. Alternative strategies for live-stock-fish integration with emphasis on Asia. *Ambio* 28, 118-124.
- Lomovskaya, O., Lewis, K., 1992. Emr, an Escherichia coli locus for multidrug resistance. *Proceeding of the National Academy of Sciences of the United States Of America* 89 (19) 8938-8942.
- Lu, J., Sanchez, S., Hofacre, C., Maurer, J.J., Harmon, B. G., Lee, M.D., 2003. Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. *Applied and Environmental Microbiology* 69, 901-908.
- Macklin, K.S., Norton, R.A., McMurtrey, B.L., 1999. Scratches as a component in the pathogenesis of avian cellulitis in broiler chickens exposed to cellulitis origin Escherichia coli isolates collected from different regions of the US. *Avian Pathology* 28, 573-578.
- Marcucci, M.C., 1995. Propolis: Chemical composition, biological properties and therapeutic activity. *Apidologie* 26, 83-99.
- Mead, G.C., 2000. Prospects for "competitive exclusion" treatment to control salmonellas and other food-borne pathogens in poultry. *Veterinary Journal* 159, 111-123.
- Merk, 1998. *Gıda Mikrobiyolojisi*. Ankara, 67s.
- Montrose, M.S., Shane, S.M. and Harrington K.S., 1985. Role of litter in the transmission of Campylobacter jejuni. *Avian Diseases* 29, 392-399.
- Moore, Jr. P. A., Daniel, T.C., Sharpley, A.N., Wood, C.W., 1995. Poultry manure management: environmentally sound options. *Journal of Soil and Water Conservation* 50, 321-327.
- Nikaido, H., 1999. Microdermatology: Cell Surface in the Interaction of Microbes with the External World. *Journal of Bacteriology* 181(1), 4-8.
- Nováková, I., Kačániová, M., Haščík, P., Fikselová, M., Močár, K., Liptaiová, D., Štofán, D., Pavličová, S., Trakovická, A., 2008. Účinok podávania probiotík na podporu mikroflóry gastrointestinálneho traktu výkrmových kurčiat. In *Bezpečnosť a kvalita surovín a potravín*, Nitra: SPU, pp. 398-402.
- Petersen, A., Andersen, J.S., Kaewmak, T., Somsiri, T., Dalsgaard, A., 2002. Impact of Integrated Fish Farming on Antimicrobial Resistance in a Pond Environment. *Applied and Environmental Microbiology* 68, 6036-6042.
- Rahmani, H.R., Tabatabaei, A., Edriss, M.A., 2006. Microbial population of broiler's ileum is affected by oil extracted propolis (OEP). *European Symposium on Poultry Nutrition 15th*, pp. 327-329
- Sforcin, J.M., Fernandes, A.J., Lopes, C. A., Bankova, V., Funari, S.R., 2000. Seasonal effect on Brazilian propolis antibacterial activity. *Journal of Ethnopharmacology* 73, (1-2) 243-249.
- Soerjadi, A.S., Snoeyenbos, G.H., Weinack, O.M., 1982. Intestinal colonization and competitive exclusion of Campylobacter fetus subsp. jejuni in young chicks. *Avian Diseases* 26, 520-524.
- Takaisi-Kikuni, N.B., Schilcher, H., 1994. Electron microscopic and microcalorimetric identified possibility of how bee propolis antibacterial effect works in a defined condition. *Planta Medica* 60 (3), 222-227.
- Tannock, G.W., 1988. The normal microflora: new concepts in health promotion. *Microbiology Science* 5, 4-8.
- Tegos, G., Stermitz, F.R., Lomovskaya, O., Lewis, K., 2002. Multidrug Pump Inhibitors Uncover Remarkable Activity of Plant Antimicrobials. *Antimicrobial Agents and Chemotherapy* 46 (10), 3133-3143.
- Tekeli, A., Kutlu, H.R., Celik, L., Doran, F., 2010. Determination of the Effects of Z. officinale and Propolis Extracts on Intestinal Microbiology and Histological Characteristics in Broilers. *International Journal of Poultry Science* 9, 898-906.
- Tosi, A.E., Re' E., Ortega, M.E., Cazzoli, A.F., 2007. Food preservative based on propolis: bacteriostatic activity of propolis polyphenols and flavonoids upon Escherichia coli, *Food Chemistry* 104, 1025-1029.
- Van der Wielen, P.W. J.J., Keuzenkamp, D.A., Lipman, L.J. A. van Knapen, F., Biesterveld, S., 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. *Microbiology Ecology* 44, 286-293.
- Vidanarachchi, J.K., Mikkelsen, L.L., Sims, I.M., Iji, P.A., Choct, M., 2006. Selected plant extracts modulate the gut microflora in broilers. *Australia Poultry Science Symposium* 18, 145-148
- Weinack, O.M., Snoeyenbos, G.H., Smyser, C.F., Soerjadi, A.S., 1981. Competitive exclusion of intestinal colonization of Escherichia coli in chicks. *Avian Disease* 25, 696-705.
- Weinack, O.M., Snoeyenbos, G.H., Soerjadi-Liem, A.S., Smyser, C.F., 1985. Therapeutic trials with native intestinal microflora for Salmonella typhimurium infections in chickens. *Avian Disease* 29, 1230-1234.
- Willis, W. L., Murray, C., Talbott, C., 2002. Campylobacter isolation trends of age versus floor broiler chickens: a one way study. *Poultry Science* 81, 629-631.
- Zgurskaya, H.I., Nikaido, H., 1999. By passing the periplasm: Reconstitution of the AcrAB multidrug efflux pump of Escherichia coli. *Proceeding of the National Academy of Sciences of the United States of America* 96(13), 7190-7195.
- Zinedine, A., Faid, M., 2007. Isolation and Characterization of Strains of Bifidobacteria with Probiotic Properties In vitro. *World Journal of Dairy and Food Sciences* 2, 28-34.