Advances in Animal and Veterinary Sciences 2 (4): 204 – 211
http://dx.doi.org/10.14737/journal.aavs/2014/2.4.204.211

Research Article

Influence of Propolis on Intestinal Microflora of Ross Broilers Exposed to Hot Environment

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ARTICLE HISTORY

Received: 2014–03–11
Revised: 2014–03–23
Accepted: 2014–03–23

Key Words: Propolis, Heat stress, Lactobacilli, Total aerobics, Coliforms, Litter

ABSTRACT

The negative impact of summer temperature in Egypt is an issue of great concern in poultry health and production. In this regard, the possibility of using ether extract propolis (EEP), as a natural additive, in broilers diets exposed to high environmental temperature is being researched. Lactobacilli spp. and bifidobacteria as well as total aerobic and coliform bacterial count in heat– stressed broiler chicks were determined. Effect of EEP on total aerobic and coliform bacteria in litter, kept under high environmental temperature, was also assessed. Broilers were held at 38±1.4°C from day 15 till the end of the study at day 42. During the study, broilers (Ross 308) were distributed to four dietary treatments; a control diet and three diets containing 250, 500 and 750 mg/kg diet EEP. Results revealed that propolis might alleviate hypothalamic–pituitary–adrenal (HPA) axis response induced by heat stress through increasing both lactobacilli and bifidobacteria and reducing total aerobics and coliform bacteria within the gut of broiler chicks. In addition, the positive effect of EEP in suppressing the total aerobic and coliform bacterial counts in broilers litter has been clearly shown at the end of the experiment.

ARTICLE CITATION: Abdel–Mohsein HS, Mahmoud MAM, Mahmoud UT (2014). Influence of propolis on intestinal microflora of ross broilers exposed to hot environment. Adv. Anim. Vet. Sci. 2 (4): 204 – 211.

INTRODUCTION

Summer temperature is an issue of great concern, since its variability and extremes have important economical and health implications on poultry production. Egypt has a hot desert climate. Peak temperatures in Upper Egypt (southern parts of Egypt) are from May to October, which is usually compensated by extremely low humidity, although in the last few years the humidity has spread. In summer days, the temperature reaches 45°C and the performance of birds is accordingly reduced drastically. The suitable temperature for poultry breeding is between 16 and 25 ◦C (Filizciler et al., 2002; Cerci et al., 2003; Sahin et al., 2006). High ambient temperatures coupled with high humidity are critical on poultry health. Exposing broiler chickens to continuously high temperature especially during the finisher phase leads to chronic heat stress (Sahin et al., 2003; Ahmad et al., 2008). Profound effect on overall physiology and animal health, which can lead to changes in body composition, could be resulted from heat stress (Akbarian et al 2013).

Heat stress is characterized by endocrine disorders, reduced metabolic rate, lipid peroxidation, decreased feed consumption, decreased body weight gain, higher feed conversion ratio, immune–suppression, and intestinal microbial dysbiosis (May et al., 1986; Lan et al., 2004; Sansonetti, 2004; Sohail et al., 2010, 2011). Increased intestinal permeability, altered morphology, as well as changes of the microbial community structure in the intestinal tract of broilers subjected to heat stress has been reported (Burkholder et al. 2008 and Garriga et al. 2006). Exposure to extreme temperature is also associated with increased intestinal colonization and fecal shedding of pathogens in poultry (Bailey, 1988). Hormonal changes during stress affect epithelial mucus secretion and mucus composition as well as the acid–base balance in the gastrointestinal tract. These conditions disturb the balance of the microbial population of the intestine by species rearrangements, ecological barrier disorders, and the colonization of pathogens in gut (Tannock and Savage, 1974; Lizko, 1987; Rowland, 1988; Tannock, 1988; Fuller, 1989; Barrow, 1992), Alterations in the mucus layer could change the attachment capabilities of both commensal and pathogenic microorganisms (Deplancke and Gaskins, 2001).

The intestinal microflora provide a 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). Some of the intestinal flora, as lactobacilli and bifidobacteria, can compete with pathogens, maintain intestinal immune homeostasis and prevent inflammation and release

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

bacteriocidal or bacteriostatic chemicals (Powrie, 1995; Cebra, 1999 and Lan et al., 2005).

Intervention strategies to deal with heat stress conditions have been the focus of many published studies, which apply different approaches, including environmental management, nutritional manipulation as well as inclusion of feed additives in the diet (e.g., antioxidants, vitamins, minerals, probiotics, prebiotics, essential oils, *etc.*) (Lucas et al. 2013). Propolis, natural feed additives, is an adhesive, dark yellow to brown colored balsam that smells like resin. It is collected from buds, leaves and similar parts of trees and plants like pine, oak, eucalyptus, poplar, chestnut, *etc.* by bees and mixed with wax (Valle, 2000). Antibacteial, antioxidative, cytostatic, antimutagenic and immunomodulatory properties of propolis are based on its rich, flavonoid, phenolic acid and terpenoid contents (Kimoto et al., 1999 and Prytzyk et al. 2003). Propolis exhibits bacteriostatic activity against different bacterial genera and can be bactericidal at high concentrations (Drago et al. 2000).

In chicken production, litter is a potential reservoir and transmission vehicle for pathogens and potential pathogens (Lu et al. 2003, Montrose et al. 1985, Weinack et al. 1985, Willis et al. 2002). Litter microbiological quality can help poultry producers to reduce infection, improve production and performance and lower costs in chicken. Some work has been done to study the effect of litter quality on the poultry health in attempt to reduce the presence of pathogenic bacteria and improve the environmental conditions of the chicken houses (Tollba and Mahmoud 2009, Fernanda 2006). It is reasonable to speculate that high environmental temperature would not only affect the bacterial level in the feces of birds, but also the duration and contamination level in the environment (Lara and Rostagno, 2013). Environmental stress has been shown to be a factor that can lead to colonization of farm animals by pathogens, increased fecal shedding and horizontal transmission, and consequently, increased contamination risk of animal products (Rostagno 2009 and Verbrugghe et al. 2012). Poultry litter has been traditionally applied to agricultural soils for decades as an organic fertilizer, because it is good source of plant nutrients (Moore et al., 1995). In addition, litter is extensively used as fertilizer in fish ponds or as fish food in Egypt. Using of litter or poultry manure as a cheaper alternative for artificial fertilizer to increase phytoplankton production (natural fish food) in fish ponds are common from different countries (EAHMI 2008; Petersen et al. 2002; Little and Edwards 1999 ; Knud–Hansen 1993). Reduce bacterial load in litter could enhance a safe use of their nutrients in agricultural and non–agricultural application.

Numerous techniques have been proposed as possible therapies to offset the consequences of heat stress. In this regard, Dietary manipulation of natural additives in animal diets could be a feasible option. Propolis is available at low cost in most seasons. Dietary ethanol extracts of propolis supplementation improved growth, performance and carcass qualities in broilers under heat stress (Tatli Seven et al. 2008). However, according to our best knowledge, there is no available data about the effect of propolis on the intestinal microflora under heat stress. It was expected that selected cheap natural feed additive as propolis would normalize the deleterious effects of heat stress on broiler chicks. Therefore, the current preliminary study aims to determine the effect of dietary propolis supplementation on fecal and litter bacterial population of broiler chickens exposed to high ambient temperature.

Materials and methods

Birds and Treatments:

A total of Sixty four, one–day–old broiler chicks (Ross 308) were used in the study. The chicks were randomly assigned, according to their initial body weights, to 4 treatment groups, 16 birds each. The experiment was planned in accordance with animal welfare. All pens were bedded with a wood–shavings litter and equipped with feeders and waterers in environmental chambers. Birds were reared at a density of 20 kg/m^2. Continuous lighting program (23 hours lightning and 1 hour darkness) was applied. Birds were given a starter diet to 21 days of age followed by a grower diet till the end of experiment. The ingredients of the diet and calculated energy uptake/ Kg diet are presented in Table 1. The feed was administered ad libitum, the starter and grower diet were the same for all the 4 treatments, and formulated according to the recommendations of the National Research Council of the US (NRC, 1994). Via their drinking water chicks were vaccinated against New Castle Disease at days 6, 14, 21, and 32 and against Infectious Bursal Disease (IBD) at days 10, 18 and 25.

Table 1: Composition of the experimental diets (%)

Ingredients	Starter	Grower
corn	50.5	60.05
Fish meal	3.5	3
SBM	36.75	29
Sunflower oil	6	4.7
Dicalcium phoshate	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 nutrient content	Starter	Grower
ME (Kcal/kg)	3202.465	3208.048
CP (%)	22.977	20.0363

During the experimental period (15–42 days of age), the four groups were kept under 38±1.4°C and 49±2% RH. The heat source was provided by electrical heaters. Chicks under heat stress treatments were fed as follow: 1. Basal diet, no additives (Control), 2– Basal diet + 250 mg EEP/kg feed 3– Basal diet + 500 mg EEP /kg feed, 4– Basal diet + 750 mg EEP /kg feed. The addition of EEP to the diet was started at day 15. EEP was purchased from Dalian Tianshan Industrial Co. ™, Ltd. Changjiang Road, Dalian, Liaoning, and China.

Sampling and Bacteriological Analysis

Fecal and litter samples were collected from each group at days 7 and 27 post treatment for bacteriological examination. Fresh fecal samples from birds were aseptically collected on a sterile sheet and immediately transferred to sterile plastic bags. Litter samples were taken from each group using sterile plastic bags. Samples were 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 64 fecal and 24 litter samples. The fecal and litter samples were accurately weighed and diluted in sterile saline to an initial 10–1 dilution. Most Probable Number method (MPN), using

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

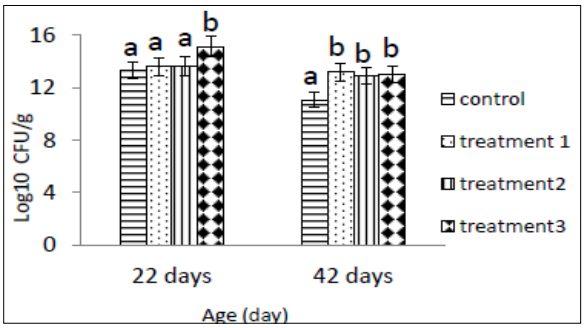
MacConkey broth (Lab M Limited, Lancashire BL9 6As, UK), was used for coliform counts in fecal and litter samples (Halkman et al.1994). Serial dilutions were made using sterile saline solution (0.85%) for total aerobic bacterial enumeration in litter samples. One mL, from each dilution, was inoculated in duplicate on Standard plate count agar (Lab M Limited, Lancashire BL9 6As, UK) and incubated at 37°C for 24 hrs for total aerobics in litter. From fresh fecal samples, tenfold dilution was spread in duplicate onto the following plates: Standard plate count agar, MRS Agar (pH 5.4) and MRS Agar (Biolife, Milan, Italy) supplemented with 0.25% L–cysteine hydro–chloride for total aerobes, Lactobacilli spp. and bifidobacteria, respectively. Plate count agar plates were incubated aerobically at 37 °C for 24 hrs. MRS Agar and MRS Agar supplemented with L–cysteine hydro–chloride agar plates were incubated anaerobically (Gas–Pak anaerobic system, Becton Dickinson Microbiology Systems) at 37^0 C for 48 hrs (Zinedine and Faid, 2007 and Asperger and Saad 1999). Counts were made and recorded as total aerobes, Lactobacilli and bifidobacteria colony forming units per gram of fecal at each incubation period and expressed as log10 cfu g–1.

Statistical Analysis

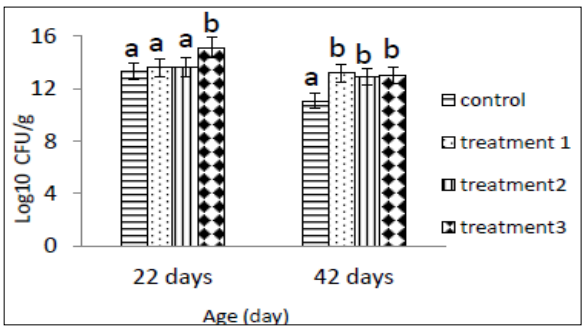
Statistical Analysis of data was carried out using SPSS version 11 statistical package programs. A one– way analysis of variance (ANOVA) was performed. Differences in mean values were accepted as being statistically significant if P< 0.05. When the effect was significant (p<0.05), means were separated using Tukey's test.

RESULTS

Effect of dietary supplementation with EEP on bacterial counts in fresh fecal samples of broiler chickens subjected to chronic heat stress are shown in figure 1. The positive effect of dietary EEP supplements on total aerobics, *Lactobacillus* spp. and Bifidobacterium appear clearly by the prolonged administration to chicks subjected to heat stress. The data about lactobacilli and bifidobacteria count in fecal samples is presented in Figure 1 (a & b). Lactobacilli and bifidobacteria counts in samples collected 7 days post treatment showed no or a slight increase except heat stress group, received 750 mg EEP /kg diet, showed a highly significant increase (P=0.002) in *Lactobacillus spp.* counts. Fecal samples collected 27 days post treatment, showed a highly significant (p<0.01) increase in lactobacilli count in all groups received different EEP concentrations. Dietary EEP increased viable counts of Bifidobacterium in all heat stress groups 27 days post treatments and recorded a highly significant increase (P=0.002) at concentrations of 500 and 750 mg/kg diet. Additionally, a highly significant (p<0.01) decrease in the counts of both lactobacilli and bifidobacteria, from the control group, were observed in the fecal samples collected 27 days post heat stress exposure than samples collected after 7 days exposure (Figure 1).



1 (a)



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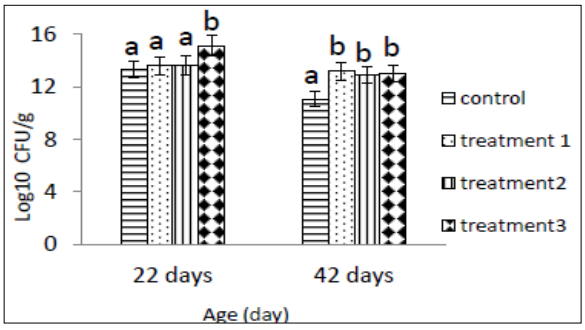

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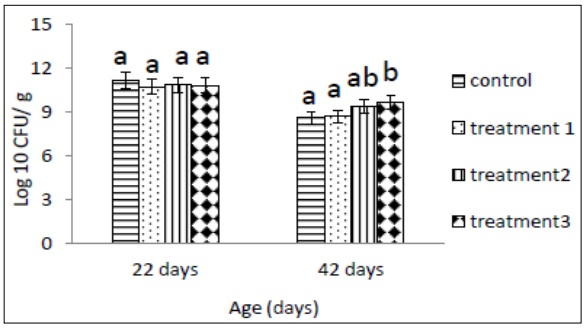


1 (d)

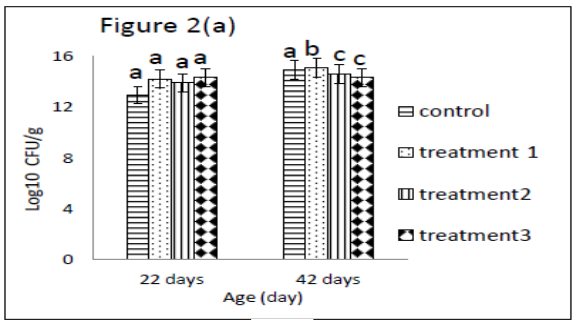
Figure 1(a, b, c and d): Log 10 CFU of the different types of bacteria in fecal samples of broiler chicken. A, b, c &d: Lactic acid, Bifidobacteria, total aerobic and colifom counts in samples collected 7 and 27 days post treatment, respectively; the groups were as follow: Control (Basal diet), Treatment 1; Basal diet + 250 mg EEP /kg diet, Treatment 2; Basal diet + 500mg EEP /kg diet, Treatment 3; Basal diet + 750 mg EEP /kg diet; abc: means with different letters are significantly different (p < 0.05)

Total aerobic and coliform counts in fresh fecal samples of broiler chickens subjected to chronic heat stress are presented in figure 1 (c & d). In this respect, feeding with EEP (250, 500 and 750 mg/kg diet) for 27 days caused a

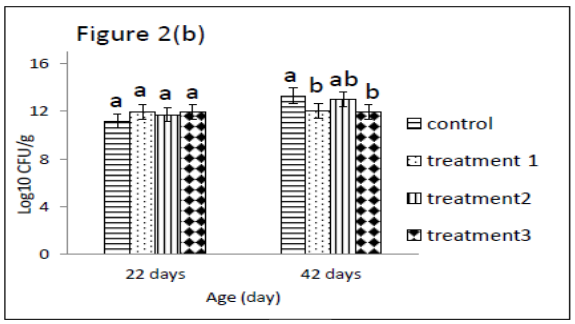
ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

highly significant decrease (P=0.001) in total aerobic counts in fecal samples compared to control group as shown in figure 1(c). Additionally, the lowest count (14.8 log10) was recorded in group received 500 mg EEP/kg feed. Fecal samples from the control group collected 27 days post heat stress exposure showed a highly significant ($p<0.01$) higher total aerobic count than that collected after 7 days exposure. The result of the total coliform count in the fecal samples is presented in Figure 1(d). No significant difference was observed in coliform count between control and EEP treated groups. However, *coliform* counts were decreased among the treatment groups at both 7 and 27 days post treatment.

Counts of total aerobics and coliform bacteria in litter samples collected at day 22 and 42 from broiler chicks subjected to chronic heat stress were presented in figure 2 (a & b). Counts pattern of total aerobic and coliform bacteria were similar among the treatment groups at days 22 and 42 of the experiment. The effect of EEP on the total aerobic and coliform bacteria was affected by the prolonged contact between the microbs and litter in the environment. Results revealed no significant differences in both total aerobics and coliform counts at day 22 between control and heat stress groups. However, at day 42 of the experiment, both counts were decreased significantly (P=0.004) among all treatment groups except the concentration of 250 mg EEP/kg diet showed no decrease in total aerobics.



A



B

Figure 2: Log 10 CFU of total aerobic and coliform count of litter samples collected at day at day 22 and 42 from of the experiment as shown in a & b respectively; the groups were as follow: Control (Basal diet), Treatment 1; Basal diet + 250 mg EEP /kg diet, Treatment 2; Basal diet + 500mg EEP /kg diet, Treatment 3; Basal diet + 750 mg EEP /kg diet; abc: means with different letters are significantly different ($p < 0.05$)

DISCUSSION

The avian gastrointestinal tract harbors a diverse and dynamic population of microorganisms, living in symbiotic relationship with their host, which is important for host nutrition, metabolism, and immunity (Sohail et al., 2010). Many stressors, including feed withdrawal, pathogenic invading and temperature fluctuations, have been reported to influence the intestinal microbiota (Burkholder et al., 2008). This implies that stress mediators can alter the mucosa–bacterial interactions and so affect the commensal microbiota and/or the outcome of a bacterial infection (Lyte et al., 2011). During stress, the release of norepinephrine from sympathetic nerves that innervate the myenteric plexus, the submucosa and mucosa of the intestine, can accelerate intestinal motility, colonic transit and transepithelial ion transport, which can influence the microbial population of the gut (Enck et al., 1989; Mizuta et al., 2006; Freestone et al., 2008).

Very little has been published on the effects of environmental stressors (particularly, heat stress) on the intestinal microbial ecosystem of poultry. Heat stress has been shown to change the microbial community structure in the intestinal tract of broilers subjected to heat stress (Burkholder et al. 2008). Therefore, it is reasonable to assume that heat stress would also affect the intestinal microbial populations of poultry (Lara and Rostagno, 2013). High environmental temperatures alter the activity of the neuroendocrine system of poultry, resulting in activation of the hypothalamic–pituitary–adrenal (HPA) axis, and elevated plasma corticosterone concentrations (Quinteiro–Filho et al. 2010 and Quinteiro–Filho et al. 2012). It was observed that stressed chicks normally exhibit elevated plasma levels of corticosterone because of high temperature (Lin et al., 2006).

Studies showed that stress can disturb the balance of the intestinal microbiota and lead to the excessive growth of pathogens while decreasing the proportion of beneficial bacteria such as lactobacilli and bifidobacteria, the two major species of the intestinal microflora (Fuller, 1999; Lan et al., 2004; Selig & Patterson, 2004; Lutgendorff et al., 2008). The relationship between the GIT microbial community, particularly the beneficial bacteria and HPA–axis development which might be disrupted by the detrimental effects of stress were clearly demonstrated by Meimandipour et al. (2010) .It has been reported that the GIT microflora cooperate in the development of the hypothalamus–pituitary–adrenal (HPA) axis. Some components of the bacterial cell wall, particularly the beneficial bacteria, may induce immune cells to secrete cytokines which consequently affect HPA–axis activity (Sudo et al., 2004).

It can be concluded that commensal bacteria, including lactobacillus and bifidobacteria has the ability to reduce the negative effect of HPA axis and corticosteron hormone level in birds. To the best of our knowledge the influence of EEP on the intestinal bacterial population and HPA axis activity induced by heat stress in broiler chickens has not been investigated. Therefore, it can be hypothesized that EEP supplementation to broilers subjected to heat stress could improve the HPA axis and reduce the blood corticosterone

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

level, through the restoration of intestinal microbial ecology. Our experiment revealed that birds exposed to high environmental temperature and given EEP exhibited a significant increase in lactobacilli and bifidobacteria count. During the same experiment, broilers subjected to heat stress and received EEP to their diets had significant low blood corticosterone concentrations compared with the control group (unpublished data). Similar to our result, some studies have been published about the positive effect of *Lactobacillus species* on stress. Experiments showed that probiotic treatment consisting of *Lactobacillus rhamnosus* and *Lactobacillus hellveticus* can ameliorate the enhanced HPA axis activity induced by maternal separation stress in rats, suggesting that probiotics normalize the activity of HPA axis (Gareau et al., 2007). Furthermore, restraint stress in germ–free mice increased the HPA–axis activity compared to conventional mice, which was reversed by the administration of probiotic bacteria to the germ free group (Sudo et al., 2004). The attenuation of the HPA axis response to stress by *Lactobacillus farciminis* depends upon the prevention of intestinal barrier impairment and decrease of circulating lipo–polysaccharides levels (Ait–Belgnaoui et al. 2012). In another study, *Lactobacillus farciminis* treatment prevents stress induced hypersensitivity and increase in colonic paracellular permeability (Ait–Belgnaoui et al. 2006).

The indigenous gut microflora is a complex ecosystem that can benefit the host by serving as a barrier to pathogen colonization (Van der Waaij, 1989). The mechanisms likely to explain the favorable effects of commensal intestinal bacteria, particularly lactobacillus and bifidobacteria to inhibit the colonization of pathogens have been enumerated in many studies. Competitive exclusion is a mechanism involving the establishment of an intestinal population of beneficial bacteria via the alteration of the intestinal pH and competition for binding sites and nutrients required for their growth with potential pathogens (Keita and So¨derholm, 2010 and Van der Wielen et al., 2002). Furthermore, lactobacilli secrete bacteriocins (Jin et al., 1996ab) and bifidobacteria produce organic acids and other bactericidal substances (Gibson and Wang, 1994); all of these substances can suppress the colonization of the intestines by pathogenic bacteria. In addition, lactobacilli have been reported to produce nitric oxide (NO), a metabolite from the metabolism of arginine, or from the reduction of nitrate and nitrite. Nitric oxide has been shown to be cytotoxic for microorganisms, help maintain an intact mucosal barrier, and reduce inflammation in the intestinal tract (Conner and Grisham, 1995; Cuzzolin et al., 1997; Dijkstra et al., 2004). Bifidobacteria have been shown to attach to cultured human intestinal epithelial cells and inhibit the attachment by enteropathogenic bacteria (Bernet et al. 1993). Lactobacilli and bifidobacteria in broilers competitively exclude gram–negative pathogenic bacteria from the intestine (Spring et al., 2000; Fernandez et al., 2002; Denev et al., 2005). Alteration of this protective barrier may leave the host more susceptible to colonization by enteric pathogens (Durant et al., 1999). Neurohormones associated with stress can induce alteration of the gut microbiota, increase growth and virulence factor expression in pathogenic bacteria within the lumen and alter host susceptibility to pathogenic bacteria and thus posing a threat to bird's health and food safety (Gaggìa et al., 2011, Keita and So¨derholm, 2010, Bailey et al., 2004 and Lyte & Bailey, 1997). Furthermore, stress can modulate the intestinal permeability and promote the luminal attachment of pathogenic bacteria (Zareie et al., 2006; Lyte et al., 2011). There is increasing evidence that stress promotes the colonization of farm animals by enteric pathogens such as *E. coli*, *S. enterica* and Campylobacter (Rostagno, 2009). Hinton et al. (2000) showed an increase in intestinal *Enterobacteriaceae* and cecal aerobes with a concurrent decrease in lactic acid bacteria in broilers subjected to a 24–h feed withdrawal. In swine heat stress results in the increased propulsion of resistant bacteria from the upper– to the lower gastrointestinal tract (Moro et al. 2000). Moreover, Heat stress significantly decreased the intestinal bacterial populations of birds (Burkholder et al. 2008). Our experiment showed decrease in total aerobic and coliform counts in broiler chicks subjected to heat stress after received EEP in their diets(although the decrease was not significant in coliform count). It can be stated that EEP has the ability to compensate or normalize the effect of heat stress on the colonization of the intestines by pathogenic bacteria. The mechanism of this action may owe to the antibacterial bioactive substance of EEP or through the activation of beneficial intestinal microbiota which competitively excluding pathogens.

Many reports indicate that propolis and its constituents protect against stress and oxidative damage. Ethanol extract propolis supplemented (dose of 3 mg/kg diet) might be considered to prevent oxidative stress in the broilers exposed to heat stress (Seven et al. 2009). The benefit of proplis to alleviate Oxytetracycline–induced oxidative stress and immunosuppression has been confirmed (Enis Yonar et al. 2011). In addition, Zhao et al. (2009) study provided evidence that propolis had therapeutic potential as hepatoprotective agent as propolis augments the antioxidants defense against mercury induced toxicity in mice.

An important measure of a suitable environment is proper maintenance of poultry litter. Very high bacterial loads could result if litter is not kept at acceptable conditions. It is reasonable to speculate that high environmental temperature would not only affect the bacterial levels in the feces of birds, but also the duration and level of contamination in the environment where feces are deposited, potentially leading to increased dissemination (Lara and Rostagno, 2013). Litter 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)

Results of litter samples revealed, decrease in total aerobic and coliform bacterial counts, at day 27 of the experiment. It can be assumed that the positive effect of EEP on the total aerobic and coliform bacteria was affected by the prolonged contact with the microbs either inside the bird or in litter. This observation is in agreement with previous reports which concluded that propolis inhibited the growth of the gram–negative photosynthetic bacteria suggesting that the effect of propolis may be species–dependent (Mirzoeva et al 1997). The obvious way to reduce *Bacterial load, total aerobics and coliform,* in the litter is to reduce the populations of these bacteria in the intestinal tracts of chickens and reduce the stressor exposure which therefore

ISSN: 2307–8316 (Online); ISSN: 2309–3331 (Print)

could reduce the incidence of disease. This observation was coincide with Estrada et al. (2001) who proposed that because feces are the main source of bacteria in poultry litter, reducing the numbers of *E. coli* present in poultry litter, might result in a lower incidence of cellulitis in broilers. Cellulitis has been associated with high concentrations of *E. coli* in the litter, which can gain entry into the skin of the chickens through abdominal scratches (Macklin et al. 1999). Also, there is increasing evidence that exposure to various stressors lead to increase fecal shedding of pathogens into the environment as shown by Verbrugghe et al. (2012).

EEP supplementation in broilers subjected to heat stress showed a significant effect on the microbial populations in the gut. The propolis activity and chemical compositions depend on plant species, season of propolis harvesting and geographical location of bee–hive collected (Bankova et al. 2000; Banskota et al. 2000). Chinese propolis is a poplar type propolis, and flavonoids, cinnamic acids and their esters are the main active components in this propolis (Bankova 2005). The total concentration of phenol, and flavonoid compounds were 19.44% and 18.792% in the EEP, respectively (Agarwal et al. 2012). Likewise, Gardana et al. (2007) found that the Chinese propolis was characterized by the presence of phenolic acids and flavonoids and the most abundant compounds are chrysin, pinocembrin, pinobanksinacetate and galangin. Presence of flavonoids and phenolic acids is found to be responsible for the antimicrobial activity of propolis (Mukherjee et al. 2006, Jasprica et al. 2007). The mechanism of action of antibacterial properties of flavonoid is by interfering bacterial cell wall permeability, microsome, and lysosome as a result of its interaction with bacterial DNA (Bryan 1982). However, due to hydrophobicity of phenolic compounds it was able to disintegrate the outer membrane of gram–negative bacteria, and disturbing the bacterial structure Liu et al. (2008). Similarly, Michiels et al. (2007) concluded that phenolic compounds can especially be used to reduce the bacterial population in the proximal and more acidic parts of the gastrointestinal tract. Variation in chemical composition due to seasonal and geographic changes brings non–significant change in their antibacterial activity. (Liu et al. 2007 and Toda and Nakanishi –Toda 2007). In our study antibacterial and anti-stress actions were observed after EEP administration to broilers. It was proposed that biological effects of propolis couldn't be attributed solely to these components, since the chemical composition of propolis is complex, (Fatoni et al. 2008). The antimicrobial properties of propolis are related to the synergistic effect of its compounds (Santos et al. 2002). Propolis affects the cytoplasmic membrane and inhibits bacterial motility as well as enzyme activity (Mirzoeva et al. 1997). Meanwhile, propolis exhibits bacteriostatic activity against different bacterial genera and can be bactericidal in a high concentration (Mirzoeva et al. 1997; Drago et al. 2000). In addition, a probable mechanism of protection against stress–mediated pathologies may be associated with the presence of flavonoids and phenolic acids were suggested by Othman et al. 2013.

CONCLUSION

EEP increased the fecal populations of lactobacilli spp. and bifidobacteria in broiler chicks exposed to chronic heat stress. In addition, results displayed that EEP was effective in suppressing the total aerobic and coliform growth in broilers exposed to chronic heat stress. The total aerobic and coliform growth in litter is reduced by the effect of EEP under high environmental temperature. The positive effects of EEP in our study were more pronounced, with increased supplement concentration and/or prolonged administration, suggesting that the action of EEP on microbes may be dose related. Therefore, under the conditions of this study, diets containing EEP (500 mg/kg diet) for 27 days offered a significant improve in the intestinal microbial ecology of broilers subjected to hot environment.

Study suggested that beneficial effects of EEP on normalizing HPA–axis activities due to heat stress seem to be dependent on increasing both lactobacilli and bifidobacteria and reducing of total aerobics and coliform bacteria within the gut of broiler chicks. Further studies are required to fully explore dose response effects and regimen of EEP on intestinal microflora and antioxidant activities as feed additive ameliorate heat stress in broilers.

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