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Effect of Dietary Propolis and Frankincense on Some Microbial Traits of Broiler

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ABSTRACT

This study was conducted in the Poultry Field of Animal Production Department / College of Agriculture / University of Anbar - in the alternative site (Abu Ghraib). This study lasted for 42 days. The experiment aimed to study the addition of propolis and frankincense and their mixture to the diet at levels of 0, 200, 400 and 600 mg / kg feed on the microbial traits of broiler. For this study (240) one day old unsexed broilers (ROSS 308) were used, with an average weight of 40 g. The chicks were distributed on ten treatments with three replicates per treatment (24 chicks /treatment). The total bacterial count and aerobic bacterial count were counted from chicken feces. The results of this study showed a decrease in the number of total air bacteria and the count of the colon bacteria under study by increasing the levels of propolis, frankincense and their mixture compared to control treatment. The study also showed the inhibitory effect on the count of colon bacteria and the count of air bacteria as affected by using different levels of propolis compared to frankincense and the combination between them.

INTRODUCTION

The poultry industry witnessed a great development and a huge expansion in the field of nutrition and in the field of breeding and improvement. With this development the disease problems has increased, since over the years, antibiotics have been used with poultry feed to improve growth performance and stabilize intestinal microflora to prevent the formation of pathogenic microorganisms, therefore, the increased use of these substances caused appearance of vital resistance by the pathogens, so some researchers resort to the use of natural alternatives instead of these antibiotics, including Propolis and frankincense. The propolis has discovered from 2000 years, and this important substance remained used daily by bees. The propolis is one of the natural products produced by the innovative organization of the bee hive, which is a mixture of beeswax and resin materials collected by bees from the leaf buds and phloem (السدرة، 2010). After collecting the bees for this substance, the mixture is mixed with saliva that contains amylase, which works to release glycons and flavonoids. Fernandes *et al*. (2005) noted that *Staphylococcus aureus* is more sensitive to the propolis, followed by *Salmonella* and *E. coli*. It has been observed that propolis has an enhanced effect on certain antibiotics for *Staphylococcus aureus*, especially those that interfere with protein synthesis of bacteria such as chloramphenicol, gentamycin, Vancomycin, tetracycline and Netimicin. Yaghoubi *et al*. (2007) found that the alcohol extract of propolis has a strong and effective influence especially against Gram-positive bacteria and less effect on Gram-negative bacteria. In another study in Brazil, the effect of propolis on *Staphylococcus aureus* and *E. coli* was observed to inhibit the growth of both germs in comparison to the effect of the two antibiotics Peniciline and Tetracycline (Gonsalos *et al*., 2006). Many studies have indicated the use of propolis in chicken diets to eliminate many different pathogens, whether bacterial, fungal or parasitic, especially bacteria that acquire antibiotic resistance. In addition, propolis doesn't cause any sensitivity and leaves no side effects as well as it works to kill harmful bacteria without including the bacteria found naturally in the body and this property in the propolis makes it different from antibiotics, which destroys bacteria without discrimination (Martos *et al*., 2008).

The frankincense is considered an important commercial material used as incense in the social and religious rituals in the Arabian Peninsula and North Africa. It was used recently as an antioxidant and a catalyst for the immune system and it was noted that the oils extracted from the gum tree has an anti-bacterial effect especially against the Gram-positive bacteria (Ramzi *et al*., 2011). Also, the frankincense has the effectiveness of anti-bacterial disease, including *E. coli* and *Salmonella p.* because it contains some antimicrobial compounds such as monoterpen, di-terpen and ciscoterpen, which is responsible for anti-bacterial activity (Annchim, 2007). So, for lack of studies in the area of effect of these substances in the microbial characteristics of meat breeds, this study aimed to investigate that point.

MATERIALS AND METHODS

This experiment was conducted in the field of poultry in the Department of Animal Production / Faculty of Agriculture / Anbar University - in the alternative site (Abu Ghraib) and lasted for 42 days. 240 one day old unsexed ROSS 308 chicks with an average weight of 40 g were used in this study. The chicks was distributed on ten treatments and three replicates per treatment (24 chick / treatment). The chicks were fed on starter, grower and finisher diets as shown in Table (1).

The treatments were as follows :

1- T1 control treatment free of any additional.
2- T2 treatment control diet with the addition of propolis at level of 200 mg/kg feed.
3- T3 treatment control diet with the addition of propolis at level 400 mg/kg feed.
4- T4 treatment control diet with the addition of propolis at level of 600 mg/kg feed.
5- T5 Treatment control diet with the addition of frankincense at level of 200 mg / kg feed.

6- T6 Treatment control diet with the addition of frankincense at level of 400 mg / kg feed.
7- T7 Treatment control diet with the addition of frankincense at level of 600 mg / kg feed.
8- T8 treatment control diet with the addition of propolis at level of 200 mg/kg feed + frankincense at level of 200 mg/kg feed.
9- T9 treatment control diet with the addition of propolis at level 400 mg/kg feed + frankincense at level of 400 mg/kg feed.
10- T10 Treatment control diet with the addition of propolis at level of 600 mg/kg fodder + frankincense at the level of 600 mg/kg feed.

Table 1. Percentage and chemical composition of the starter, grower and finisher diets used in the experiment.

Ingredients %	Starter diet 1-11day	Grower diet 12-22day	Finisher diet 23-42day
Yollow corn	53	55	57
(44 %) Soybean	30	28	25
Concentrate *	5	5	5
Wheat	10	10	10
Vegetable oil	1	1	2
Limestone	0.7	0.7	0.7
Common salt	0.3	0.3	0.3
Total	100	100	100
Chemical composition**			
Metabolizable energy (ME Kcal/kg)	3015	3033	3117
% Protein	22.1	21.3	20.00
Energy/protein ratio	136.4	142.4	155.8
% Lysine	1.25	1.20	1.11
% Cystine +Methionine	0.86	0.84	0.81
% Clacium	0.61	0.61	0.60
%Available phosphorus	0.36	0.35	0.35

*** The Brocorn-5 special W is produced by (ALBLASSERDAM HOLLAND WAFI B.V.) which contains 40 % raw protein, 5% raw fat, crude fiber 2,20 %, moisture 7,13 %, ash 28,32, calcium 4,50 %, phosphorus 2,65 %, available phosphorus 4,68 %, lysine 3,85 %, methionine 3,70 %, methionine + cystine 4,12 %, tryptophan 0.42 %, threonine 1,70 %, Assimilated energy 21.07, Selenium 2.30 % and Copper 4 %.**

**** According to the values of chemical composition by N.R.C (1994).**

Chicks were fed on starter, grower and finisher diets as shown in Table (1) and the health and preventive program recommended by the veterinarian specialist has followed during the duration of the experimental time. Propolis and frankincense were obtained from the local market of Ramadi town.

The bacterial study was carried out in the microbiology laboratory of the Department of Food Science at the Faculty of Agriculture / Anbar University (the alternative site - Abu Ghraib). The feces samples were used to perform the total bacterial count as well as the colon bacterial count by using the decimal dilutions of the samples and N.Agar and MaConnky Agar, then incubated at 37 ° C for 24 hours and the total bacterial count and colon bacterial count were estimated (Cruickshank *et al*., 1975).

Statistical analysis :

Statistical analysis was carried out using the SAS (2001) statistical program. Significant differences between mean values were tested using Duncan's test (1955) multimode test at the mean of 0.05 and 0.01.

RESULTS AND DISCUSSION

Table (2) shows the effect of propolis addition at different levels to the diet on the total bacterial count as well as the number of colon bacteria taken from feces, where there is a significant decrease in the total number of air bacteria and coliform bacteria compared to control treatment with increasing the propolis addition level.

Kumar *et al*. (2008) found that the propolis alcohol extract has a wide effect against the Gram positive bacteria group (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and specific efficacy against gram negative bacteria such as *E. coli*, Pseudomonas, *Klebsiella pneumonia* and *Proteus mirabilis*. In addition, Bankova *et al*. (2000) reported that the propolis effect on microbial growth may be due to the association of phenolic acid with other compounds present in the cell which act to bind with the enzymatic system of the respiratory pathways in the bacterial cell by inhibiting the action of these enzymes and the other reason that the smell of the propolis is highly effective inhibition against bacterial activity. Similarly, Seven and Ismail (2008) mentioned that the propolis has a fatal effect of bacteria in the laboratory and in the organism also has a stimulating effect of liver cells to manufacture proteins necessary for the body and have a role in attacking and analyzing pathogenic bacteria, also they found that the mixing of propolis with the antibiotic (Streptomycin) had a big effect against Staphylococcus aureus bacteria. Hegazi *et al*. (2000) reported that the Propolis contains a number of natural antibiotics, especially Galangin which when taken, no side effects appear and then it excluded naturally from the body without any effect on the organisms living naturally in the intestine. A study was also conducted to compare the effect of various antibiotics and propolis against 8 bacterial isolates. The isolates showed a difference in antibiotic resistance, whereas the propolis showed a highly efficacy against most studied isolates with less concentrations than the antibiotic concentrations used in the experiment (الفدعم, 2005).

Hegazi and Abd El-Hady (2001) studied the effect of propolis alcohol extract with antibiotics such as Tetracycline and Ketoconazole on the growth of *Staphylococcus aureus* and *Escherichia coli* and showed that the propolis alcohol extract has more effective inhibition compared to the antibiotics used in the experiment. Also, Hegazi and Abd El-Hady (2002) studied the antimicrobial efficacy of two samples of Egyptian Propolis. The results showed that the two samples were effective against bacteria and yeast where the first sample was effective against *Staphylococcus aureus* and *Candida albicans*, whereas the 2nd sample showed highly effective against *Escherichia coli*

bacteria only, and this attributed to the difference in chemical composition between the two samples.

In a subsequent study, different samples of propolis were collected from Egypt, Albania, Austria, Bulgaria, France, Germany, Mongolia and the UK. They tested their effectiveness against Staphylococcus aureus, E. coli and Candida yeast. All samples showed efficacy against test microorganisms but efficacy was different depending on the origin of the propolis (Amir *et al*., 2010).

Table 2. Effect of the addition of propolis to the diet at different levels on the total bacteria count and colon bacteria.

Treatment	Total bacterial count cell/g	bacterial Colon count cell/g
Control	$3.92 \pm 99\times10^9$	$3.64 \pm 110\times10^9$
Propolis at 200mg/kg	$3.87 \pm 57\times10^9$	$2.48 \pm 32\times10^9$
Propolis at 400mg/kg	$3.74 \pm 33\times10^9$	$2.70 \pm 25\times10^9$
Propolis at 600mg/kg	$3.54 \pm 10\times10^9$	$2.50 \pm 6\times10^9$

Data in Table 3 showed the effect of adding frankincense at different levels to the diet of chicks on the total bacterial count as well as the colon bacterial count, since there was a significant decrease in the count of these bacteria under study as the concentration of frankincense added to the diet increased. Annchim (2007) reported that gum tree contains compounds (diterpene, incensol, incensol acetate and acetate) which act against various microorganisms, including fungi and strains of Gram positive and negative bacteria.

Salman *et al*. (2013) noted that using frankincense alcoholic extract produced from *Boswellia carterii* plant against two types of Gram positive bacteria, 24 isolates of *Staphylococcus aureus*, 18 isolates of Streptococcus, two types of Gram negative bacteria, 35 *Escherichia* coli isolates and 20 Salmonella pyrogen isolates and used five concentrations of gum tree extract (25, 200, 100, 75, 50 mg / ml). The extract of frankincense had an antibacterial effect as S. pyogen bacteria has been influenced by frankincense alcohol extract at a concentration of 200 mg/ml, while E. coli was more affected by the lowest concentration, whereas other bacterial species increased their susceptibility with increased concentrations and Salmonella p. was the most affected in the top concentration. Ramzi *et al*. (2011) observed that the oils extracted from the gum tree consist of monoterpene, hydrocarbons, pinene, alpha, oxygenated monoterpenes, alpha-thujene, camphor and incensol. These compounds have anti-bacterial effect especially against Gram positive bacteria when used in a quantity of 1.8-17.2 mg/ml. While, the phenolic compounds inhibit the growth and production of bacterial toxins through their non-inverse interaction with amino acids and multiple peptides of the bacterial cell wall (Tsuchiya *et al*., 2003).

Data presented in Table 4 showed the effect of addition of propolis and frankincense at different levels to the diet of chicks on the total bacterial count and colon bacterial count. Since, the studied number of bacteria was significantly decreased due to the increase in the levels of the mixture. So, the reduction of total bacterial and coliform bacteria has a significant effect on the microbial and health characteristics of meat broiler.

Table 3. The effect of adding frankincense at different levels to the diet on the total bacterial count and colon bacterial count.

Treatment	Total bacterial count cell/g	Colon bacterial count cell/g
Control	$3.96 \pm 120\times10^9$	$2.57 \pm 115\times10^9$
Frankincense at 200 mg/kg	$3.68 \pm 78\times10^9$	$2.49 \pm 20\times10^9$
Frankincense at 400 mg/kg	$3.92 \pm 36\times10^9$	$2.66 \pm 16\times10^9$
Frankincense at 600 mg/kg	$3.40 \pm 6\times10^9$	$2.55 \pm 5\times10^9$

The phenolic acids with high biological efficacy in propolis are strong oxidizing compounds such as Caffeic Acid, and the phenol toxicity of microorganisms is due to enzymatic inhibition by the oxidation compounds in propolis (Cowan, 2000). Basar (2005) revealed that gum tree contains many effective phenolic compounds such as monoterpenes, diterpenes and sesquiterpenes. These differences between higher and less inhibitory effect can be explained as pathogens are sensitive to the concentration of effective compounds in propolis and frankincense under study, thereby increasing the concentration of active compounds inhibitory of bacteria directly contributes to the improvement in the health of the chicks, which reflected its impact in increasing production and economic performance of broiler meat (النايف والنعيمى, 2015).

Table 4. The effect of adding propolis and frankincense at different levels to the diet on the total bacterial count and colon bacterial count.

Treatment	Total bacterial count cell/g	Colon bacterial count cell/g
Control	$3.87 \pm 98\times10^9$	$2.52 \pm 100\times10^9$
Propolis at 200 mg/kg + Frankincense at 200 mg/kg	$3.99 \pm 122\times10^9$	$3.21 \pm 40\times10^9$
Propolis at 400 mg/kg + Frankincense at 400 mg/kg	$3.96 \pm 100\times10^9$	$2.62 \pm 10\times10^9$
Propolis at 600 mg/kg + Frankincense at 600 mg/kg	$3.87 \pm 79\times10^9$	$2.67 \pm 2\times10^9$

REFERENCES

السدرة، زياد طارق. 2010. تأثير العكبر العراقي (Iraqi propolis) في بعض أنواع الخمائر المسببة لتلف عصائر الفواكه. مجلة ديالى للعلوم الزراعية 6 (4):227-239.

الفدعم. انفال شاكر متعب. 2005. تأثير مستخلص العكبر على نمو بعض أنواع البكتريا والمبيضات. رسالة ماجستير. بغداد – الجامعة المستنصرية.

سلمان ,عمار حقي .2009. تقييم فعالية المستخاص الكحولي للصمغ المنتج من نبات Boswellia carteri كمضاد بكتيري لبعض أنواع البكتريا السالبة والموجبة لصبغة كرام . مجلة الأنبار للعلوم البيطرية، 2 (2): 110-114 .

النايف ، حسام حكمت نافع والنعيمي ، مصطفى مؤيد حسين .2015. تاثير اضافة العكبر (صمغ النحل) والكندر (اللبان الشجري) وتوليفاتهما الى العليقة في الصفات الانتاجية لفروج اللحم . مجلة الانبار للعلوم الزراعية العدد 2 (13) :88-99.

Amir, T.; Abdolghaffar, O. and Ali, M. (2010). In vitro antibacterial activities of ethanol extract of Iranian propolis (EEIP) against fish pathogenic bacteria (*Aeromonas hydrophila*, *Yersinia ruckeri* & *Streptococcus iniae*). Brazilian J. of Microbiol., 41(4): 1086-1092.

Annchim, R. (2007). Chemical composition and antimicrobial activity of some oleogum resin essential oils from Boswellia spp., 97(9):837-844.

Bankova, V.S.; De Castro, S.L. and Marcucci, M.C. (2000). Propolis: Recent advances in chemistry and plant origin. Apidologie; 31(1): 3-15.

Basar, S. (2005). Phytochemical investigations on Boswellic species: Comparative studies on the essential oils, Pyrdystes and boswellic acids of Boswella Carterii Birdw., Boswella Serrata Roxb., Boswella Frereana Birdw., Boswella neglecta S. Moore and Boswellia Rivae, PhD Thesis, University of Hamburg, England.

Cowan, M.M. (2000). Plant Products as antimicrobial agents. Clinical Microbiol., Rev., 12(4): 564-582.

Cruickshank, R.; Dugid, P.; Marmian, B.D. and Swain, R.H.A. (1975). Medical Microbiology 12 ed, 11 Edinbury: Churchill Livingastone, London.

Duncan, D. (1955). Multiple rang and multiple F. Test, Biometrics, 11: 1- 24.

Ferrnandes Junior, A.; Leomil, L. and Fernandes, A.A.H. (2005). The antibacterial activity of propolis produced by Apis mellifera L. and Brazilian stingless bees. J Venom Anim Toxins 7: 173-182.

Gonsales, G.Z.; Orsi, R.O.; Fernandes Junior, A.; Rodrigues, P.I. and Funari S.R.C. I. (2006). Antibacterial activity of propolis collected in different regions of Brazil. *J. Venom.* Anim. Toxins incl. Trop. Dis vol.12(2): 276-284 Botucatu Apr./June.

Hegazi, A.G. and Abd El Hady, F.K. (2001). Egyptian Propolis: 1- Antimicrobial activity and chemical composition of Upper Egypt propolis. Proceedings of the 37th Inter. Agric. Cong., 28 October – 1 November 2001, Durban, South Africa.

Hegazi, A.G. and Abd El Hady, F.K. (2002). Egyptian propolis: 3- Antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands. Z. Naturforsch C. Mar-Apr; 57(3-4): 395-402.

Hegazi, A.G., Abd El Hady, F.K., Abd Allah, F.A. (2000): Chemical composition and antimicrobial activity of European propolis. Z. Naturforsch. C 55, 70-75.

Kumar, N.; Ahmad, M.K.K.; Dang, R. and Husain, A. (2008). Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone. J. of Med. Plants Res., 2(12): 361-364.

Martos, M.V.; Navajas, Y.R.; Lopez, J.F. and Alvarez, J.A.P. (2008). Functional properties of honey, propolis and royal jelly. Journal Food Science, 73(9): 117-124.

National Research Council (NRC) (1994). Nutrient requirement of poultry. 9th ed. National Academy press, Washington.

Ramzi, A.; A. Mothana; S. Hasson; D. WulfSchultze; M. Annette and L. Ulrike (2011). Phytochemical composition and in vitro antimicrobial and antioxidant activities of essential oils of three endemic Soqotraen Boswellia species Food Chemistry, 126: 1149-1154.

Salman A.F.; Payam, M.; Ali, A.; Hajar, B.M.B.; Naser, T. and Houshang, M. (2013). The effect of *Boswellia serrata* on neurorecovery following diffuse axonal injury. Brain Inj., 27(12): 1454-60.

SAS (2001). SAS/TAT user's Guide Version 6. 4th ed. SAS Institute Inc.

Seven, P.T. and Ismail, S. (2008). Effect of dietary Turkish propolis as alternative to antibiotic on performance and digestibility in broilers exposed to heat stress. J. Appl. Anim. Res., 34: 193-196.

Tsuchiya, H.; Sato, M.; Miyazaki, T.; Fujiwara, S.; Tanigak, S.; Ohyama , M.; Tanaka, T. and Linuma, M. (2003). Comparative study on the antibacterial activity of phytochemical flavonones against methicillin – resistant *Staphylococcus aurens*. J. Ethropharmacol. 50, 27-34.

Yaghoubi, S.M.J.; Ghorbani, G.R.; Soleimanian Zad, S. and Satari, R. (2007). Antimicrobial activity of Iranian propolis and its chemical composition. DARU Vol.15(1): 45-48.

تأثير إضافة صمغ النحل واللبان وخليطهما إلى العليقة على بعض الصفات الميكروبية لفروج اللحم

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أجريت هذه التجربة في مزرعة الطيور الداجنة التابع لقسم الإنتاج الحيواني /كلية الزراعة /جامعة الانبار– في الموقع البديل (أبي غريب) واستمرت هذه الدراسة لمدة 42 يوم. إستهدفت التجربة دراسة تأثير إضافة صمغ النحل واللبان وخليطهما الى العليقة بمستويات 0 ، 200 ، 400 ، 600 ملغم/ كغم علف على الصفات الميكروبية لفروج اللحم ٠استعمل في هذه الدراسة (240) فرخ لحم غير مجنس من نوع (ROSS 308) بعمر يوم واحد وبمتوسط وزن40 غرام ٠ تم توزيع الأفراخ على عشرة معاملات وبواقع ثلاث مكررات متساوية لكل معاملة (24 فرخ / معاملة) ، حسبت اعداد البكتريا الكلية الهوائية وبكتريا القولون من زرق الدجاج. أوضحت نتائج هذه الدراسة انخفاض في اعداد البكتريا الهوائية الكلية واعداد بكتريا القولون قيد الدراسة بزيادة مستويات صمغ النحل ، اللبان والخليط مقارنة بمعاملة الكنترول كما أظهرت الدراسة التأثير التثبيطي لاعداد بكتريا القولون واعداد البكتريا الهوائية عند استخدام مستويات مختلفة من صمغ النحل مقارنة بمعاملات اللبان والخليط.