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Influences of Dietary Supplementation of Antimicrobial Cold Pressed Oils Mixture on Growth Performance and Intestinal Microflora of Growing Japanese Quails

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ABSTRACT
This study evaluated the effect of dietary supplementation of antimicrobial cold pressed oils mixture [1:1:1 of *Nigella sativa*, cloves and rosemary (NCR)] at levels of 0.75 and 1.5 g kg^{-1} diet, on growth performance, carcass traits and some microbiological aspects for growing Japanese quails. A total number of 225 growing Japanese quails one week old were used in a complete randomized design experiment with three treatments. Final live body weight was significantly ($p<0.01$) higher by 2.89% in quails fed diet supplemented with 1.5 g NCR oil compared to those fed the control diet. Feed intake increased statistically ($p<0.01$) as the level of oil mixture supplement increased. The best feed conversion of 2.87 was associated with 1.5 g oil during period 3-6 weeks of age, whilst the control group gave the best feed conversion within the whole period (1-6 weeks of age) followed by the experimental group treated with 1.5 g NCR oil. Feeding quails on a diet enriched with 1.5 g NCR oil resulted in the best carcass percentage which increased by 3.72% compared to those fed the control diet. The populations of Total Bacterial Count (TBC), coliforms and *Escherichia coli* in ileum, ceca and feces were fewer ($p<0.05$) in quails fed diet supplemented with NCR oil mixtures (1.5 g w/w) compared with the control diet. In conclusion, the dietary supplementation of antimicrobial cold pressed oils mixture by 1.5 g kg^{-1} showed the potential to enhance the growth performance and reduce intestinal and excreta pathogenic bacteria in quails.

Key words: Antimicrobial cold pressed oils, growth performance, quail, bacterial pathogens, intestinal microflora

INTRODUCTION

Feed additives have been widely used to increase the performance of animals and are now used in poultry feeding practices extensively (Collington *et al*., 1990; Khan *et al*., 2007) not only to stimulate the growth and feed efficiency but to improve the health and performance of birds (Scott *et al*., 1982; Fadlalla *et al*., 2010; Abouelfetouh and Moussa, 2012; Gopi *et al*., 2014). The last years of research have focused on finding some additives to ensure proper growth and development, while protecting the body against diseases which may harm the health and welfare. Medicinal plants were investigated from the perspective of their use as bio-stimulator growth and replacement of antibiotics. Medicinal plants are being used in various forms and additives acting respectively at maintaining digestive balance of existing flora and a

functional role related to increasing specific enzyme secretion and an important antibacterial role (Trombetta *et al*., 2005; Boyraz and Ozcan, 2006; Ghazalah and Ali, 2008).

Cold pressed oils are obtained through pressing and grinding fruit or seeds with the use of heavy granite millstones or modern stainless steel presses, which are found in large commercial operations. Although, pressing and grinding produces heat through friction, the temperature must not rise above 120°F (49°C) for any oil to be considered cold pressed. Cold pressed oils are produced at even lower temperatures. Cold pressed oils retain all of their flavor, aroma and nutritional value. The consumption of new and improved products such as cold-pressed oils may improve human health, animal health and may prevent certain diseases (Lutterodt *et al*., 2010). Black cumin seed components have also been used to prepare functional cosmetic and dietary supplemental products. Studies were conducted on pharmacological properties of the essential oil of black cumin and thymoquinone on antioxidant activity (Burits and Bucar, 2000; Luther *et al*., 2007; Lutterodt *et al*., 2010) and antimicrobial activity (Hanafy and Hatem, 1991). Essential oils as phytogenic additives are qualified with many properties such as affecting feed consumption, enhancing digestive enzymes secretion and increasing the motility of the digestive tract in addition to improving the taste of feed, increasing organism immunity and antimicrobial properties (Akyurek and Yel, 2011; Gopi *et al*., 2014; Alagawany *et al*., 2015a, b). These are the reasons to display them as a natural growth promoters and successful replacement for antimicrobial growth promoters (Hengl *et al*., 2011).

Fixed oil from black cumin (*Nigella sativa*), clove (*Syzygium aromaticum)* and rosemary (*Rosmarinus officinalis*) are rich in linoleic and oleic acids as well as bioactive phytosterols and tocopherols (Ramadan *et al*., 2003; Ramadan and Morsel, 2002, 2006; Ramadan, 2007; Ramadan *et al*., 2006, 2007, 2010). Antimicrobial activity of natural extracts is closely linked with their polyphenolic content (Ahn *et al*., 2004; Alzoreky and Nakahara, 2003). Therefore, plant extracts rich in phenolics and other bioactive compounds may serve as potential natural antimicrobial agents (Luther *et al*., 2007).

Food-producing animals are considered to be the primary reservoir of non-typhoidal *Salmonella*, *Escherichia coli* and *Campylobacter* causing enteric infection in humans (Carattoli, 2008; Dhama *et al*., 2013). Pathogenic bacteria, such as *Salmonella* could enter the gastrointestinal tract of birds via the crop. The environment of the crop with respect to microbial composition and pH seems to be very important in relation to the resistance to pathogens. In addition, the antibacterial effect of dietary fixed oil in Japanese quail is believed to take place mainly in the upper part of the digestive tract (crop and gizzard). Thus, search for effective alternatives to antibiotics in intensive animal production is a very important topic, considering not only the impact in animal welfare but also in human health. As, a continuation of our efforts in developing compressed oils rich in health beneficial components for humans and animals, this study was conducted to evaluate the effect of dietary supplementation of antimicrobial cold pressed oils mixture [1:1:1of *Nigella sativa*, cloves and rosemary (NCR)] at levels of 0.75 and 1.5 g kg^{-1} diet w/w on growth performance, carcass traits and some microbiological aspects for growing Japanese quails.

MATERIALS AND METHODS

Oils: Cold-pressed oils (*Nigella sativa*, cloves and rosemary) were purchased in 2014 from local market in Zagazig, Egypt. The antibacterial activity of *Nigella sativa*, cloves and rosemary against *Salmonella enteritidis*, *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 was assayed by the Hole- Plate Diffusion method. Each organism was cultured to 250 mL nutrient agar. The mixture was shacked well and poured into sterile petri dishes to obtain the media. The plates were left at room temperature for solidification. The wells were made in plates by sterile cork borer (6 mm in diameter) and 150 µL of the neat undiluted cold pressed was placed into each well with sterile micropipette. The plates were left at room temperature prior to incubation till the oil diffused. The plates were incubated at 37°C for 24 h. After incubation the inhibition zones were measured in millimeters (mm).

Extraction and quantification of phenolic compounds: Aliquots of cold-pressed oil were dissolved in n-hexane (5 mL) and mixed with 10 mL methanol-water (80:20, v/v) in a glass tube for two min in a vortex. After centrifugation at 3000 rpm for 10 min, the hydroalcoholic extracts were separated from the lipid phase by using a Pasteur pipette then combined and concentrated *in vacuo* at 30°C until a syrup consistency was reached. The lipidic residue was re-dissolved in 10 mL methanol-water (80:20, v/v) and the extraction was repeated twice. Hydroalcoholic extracts were re-dissolved in acetonitrile (15 mL) and the mixture was washed three times with *n*-hexane (15 mL each). Purified phenols in acetonitrile were concentrated *in vacuo* at 30°C then dissolved in methanol for further analysis. Aliquots of phenolic extracts were evaporated to dryness under nitrogen. The residue was re-dissolved in 0.2 mL water and diluted (1:30) Folin-Ciocalteu's phenol reagent (1 mL) was added. After 3 min, 7.5% sodium carbonate (0.8 mL) was added. After 30 min, the absorbance was measured at 765 nm using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid was used for the calibration and the results of triplicate analyses are expressed as parts per million of gallic acid.

Birds and diets: The present study was carried out at Poultry Research Farm, Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. All experimental procedures were carried out according to the Local Experimental Animal Care Committee and approved by the ethics of the institutional committee of Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

A total number of 225 healthy growing Japanese quails one week old with average initial body weight of 23.67±0.05 g were used in a complete randomized design experiment with three treatments. Each group was subdivided into five replicates with 15 unsexed chicks. From one to six weeks of age, quails were fed the basal diet with or without supplemental oil mixture which formulated to meet birds' requirements according to NRC (1994). The cold pressed oils mixture consisted of equal amounts of three oils (1:1:1) of *Nigella sativa* oil, clove oil and rosemary (NCR) oil. The treatments were as follows: (1) Control basal diet, (2) Basal diet+0.75 g oil mixture/kg diet, (3) Basal diet+1.5 g oil mixture/kg diet. The ingredients and chemical composition of the basal diet are presented in Table 1. Birds were housed in conventional type cage (50×30×50 cm^3; 1,500 cm^2 of floor space) with feed and fresh water provided *ad libitum*. Birds also were maintained on a 24 h light throughout the trial. All chicks were kept under the same managerial, hygienic and environmental conditions.

Data collection: Chicks were weighed individually at weekly intervals. Mortality was recorded daily. Average daily feed intake, body weight gain and feed to gain ratio were calculated from these data by period and cumulatively. Feed wastage was recorded daily and the data were used to estimate feed consumption. At the termination of experiment, 5 growing quails (for each group) were sampled randomly for carcass evaluations at 6 weeks of age, weighed and manually slaughtered. Carcass weight (the main body, gizzard, liver, heart and other total edible parts) were determined according to Blasco *et al*. (1993). The carcasses were weighed and the weights of the liver, gizzard, heart, thigh, breast and abdominal fat were recorded and expressed as g kg^{-1} of slaughter weight. Carcass and dressed weights studied (dressed weight = carcass weight plus giblets weight)/live body weight.

Microbiological analysis: The excreta ceca and ileum as well as feces from 3 birds from each treatment samples were taken for microbiological studies after 7, 21, 42 days. The samples were kept in sterile eppendorf tubes of 5 mL and were immediately processed for microbiological investigation. Total viable bacterial count (TVC) was determined on Plate Count Agar (PCA, Difco) after 48 h incubation at 37°C. *Clostridium* spp. were detected on Tryptose Sulphite Cycloserine agar (TSC, Merck, 1.11972) after 48 h incubation at 37°C. Violet Red Bile agar (VRB, Biolife, Italy) was used for counting coliforms after 24 h incubation at 37°C.

Table 1: Composition and chemical analysis of the basal diet

Items	Basal diet
Ingredients (%)	
Yellow corn	53.03
Soybean meal (44%)	38.69
Corn gluten meal	3.20
Di-calcium	1.67
Limestone	0.81
Vit-min premix*	0.30
NaCl	0.30
DL methionine	0.11
L-lysine	0.39
Soybean oil	1.50
Total	100
Calculated analysis**	
CP (%)	24.04
ME Kcal/kg diet	2903
Ca (%)	0.85
P (Available) (%)	0.45
Lysine (%)	1.60
M+C (%)	0.88
CF (%)	3.92

*Growth vitamin and mineral premix each 2.5 kg consists of, Vitamin A: 12000,000 IU, Vitamin D3: 2000,000 IU, Vitamin E: 10 g, Vitamin K3: 2 g, Vitamin B1: 1000 mg, Vitamin B2: 49 g, Vitamin B6: 105 g, Vitamin B12: 10 mg, Pantothenic acid: 10 g, Niacin: 20 g, Folic acid: 1000 mg, Biotin: 50 g, Choline chloride: 500 mg, Fe: 30 g, Mn: 40 g, Cu: 3 g, Co: 200 mg, Si: 100 mg and Zn: 45 g, **Calculated according to NRC (1994)

Escherichia coli were counted on MacConkey agar (Oxoid, CM0007) after 24 h at 37°C. All the above plates were incubated under anaerobic conditions. The number of bacterial group was transferred to Log number before statistical analysis. The detection of *Salmonella* spp. was achieved by suspending the sample into 100 mL buffered peptone water then incubating at 37°C for 20 h. Then, 0.1 mL of each BPW medium was transferred into a culture tube containing 10 mL of Rappaport Vassiliadis (RV) enrichment broth and incubated again at 42°C for 24 h. From both steps, a portion of the sample was streaked on XLD agar (Merck, 1.05287) and incubated at 37°C for 24 h.

The lowest detection limit was 1 log CFU g^{-1}. All plates were examined for typical colony types and morphological characteristics associated to each culture medium. Presumptive colonies of the above bacteria were verified by confirmation tests. For estimated counts below the limit of detection, the Most Probable Number (MPN) technique was used. Serial dilutions of three Frather or buffered peptone water tubes of three successive dilutions were incubated at 37°C for 48 h. Buffered peptone water was used as a nonselective medium for recovering sublethally injured bacteria and for minimizing the underestimation of counts. All plates were examined for typical colony types and morphological characteristics associated to each culture medium.

Statistical analysis: The study consisted of a complete randomized design with three treatments. The number of replicates varied according to data assessed, including five for

performance and slaughter yield data as well as three for the microbiological studies of ileum and ceca contents. All data was analyzed by GLM procedures of SAS (SAS., 2004). The differences among means were determined using the post-hoc Tukey's new multiple range test. Statements of statistical significance are based on $p<0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Bioactive phenolic compounds in fixed oils: Cold-pressed seed oils may retain more natural benefits of the seeds, including natural antioxidants which are free of chemical contamination. Recently, cold-pressed edible seed oils have become commercially available. The cold-pressing procedure involves neither heat nor chemical treatments and is becoming a more interesting substitute for conventional practices because of consumers' desire for natural and safe food products during food chains. Cold pressed oils were characterized by higher levels of phenolics (3.5-3.7 g kg^{-1}). Thus, these oils may be used in different food or feed applications to provide nutrition and health benefits. Phenolic compounds have been reported to be present in vegetable oils, which is very important for the oxidative stability of the PUFA. Additionally, edible oils rich in natural antioxidants may play a role in reducing the risk of chronic diseases. Polyphenols are considered as powerful active compounds expressing strong antioxidant activities. This activity is mainly due to their redox potential, which can play an important role in adsorbing and neutralizing free radicals, quenching reactive oxygen species and chelating metals, especially iron and copper cations (Bettaieb *et al*., 2010; Rahal *et al*., 2014a).

Antibacterial activity of cold-pressed oils: Cold-pressed oils showed the best antibacterial activity against *S. aureus* with an inhibition zone of 12.3 mm, closely followed by *E. coli* and *Salmonella enteritidis* with inhibition zones of 10.5, 10.1 and 9.22 mm, respectively. The Minimum Inhibitory Concentration (MIC) against the studied bacteria was about 150-200 µg mL^{-1}. In a preliminary experiment, the concentration 0.75 and 1.5 g kg^{-1} (w/w) of oil gave a maximum inhibitory effect on bacterial pathogens while higher increases in concentration did not produce any significant changes ($p<0.05$). Therefore, these concentrations (0.75 and 1.5 g kg^{-1}) were selected in all the subsequent experiments.

Growth performance: The effect of dietary oil mixture supplement on the performance of growing Japanese quails during the experimental period is shown in Table 2 and 3. Final live body weight was significantly ($p<0.01$) higher by 2.89% in quails fed diet supplemented with 1.5 g oil mixture/kg diet compared to those fed the control diet. The effect of supplementation on body weight gain was insignificant within the first period (1-3 weeks), meanwhile it was highly significant ($p<0.01$) in the second period (3-6 weeks) and the whole experimental period (1-6 weeks of age). It is obvious that supplementing the diet with 0.75 g oil mixture/kg diet slightly depressed values of body weight gain but the higher level (1.5 g oil mixture/kg diet) improved body weight gain by 5.27 and 3.85% at the second and the whole period, respectively compared to the control. The present findings are in agreement with those reported by Hernandez *et al*. (2004) and Jang *et al*. (2004), who found that dietary feeding on essential oil extracted from medicinal plant increased the secretion of digestive enzymes, so enhanced nutrients digestibility and improved the performance for broiler. Moreover, many studies have shown positive effects of dietary essential oils single or mixture on body weight gain and recommended its usage as growth promoter

Table 2: Effect of dietary cold pressed oil supplement on live body weight and body weight gain of growing japans quail at different phases

	Live body weight (g)			Body weight gain (g)		
	1	3	6	1-3	3-6	1-6
Items	(weeks)					
0.00 (g kg^{-1} diet)	23.81	96.40	199.60^{b}	5.18	7.37^{b}	6.28^{b}
0.75 (g kg^{-1} diet)	23.63	94.08	195.48^{c}	5.03	7.24^{b}	6.14^{c}
1.5 (g kg^{-1} diet)	23.57	96.66	205.53^{a}	5.22	7.78^{a}	6.50^{a}
SEM[1]	0.05	0.85	1.52	0.06	0.09	0.05
p-value[2]	0.174	0.456	0.000	0.469	0.007	0.000

Different superscripts within 1 column are significantly different ($p<0.05$), [1]SEM: Standard Error Means, [2]Overall treatment p-value

Table 3: Effect of dietary cold pressed oil supplement on feed intake and feed conversion rate of growing japans quail at different phases

	Feed intake (g day^{-1})			Feed conversion (g feed/g gain)		
	1-3	3-6	1-6	1-3	3-6	1-6
Items	(weeks)					
0.00 (g kg^{-1} diet)	12.99^{b}	21.30^{c}	17.14^{b}	2.51	2.89^{b}	2.70^{b}
0.75 (g kg^{-1} diet)	13.01^{b}	22.98^{a}	18.00^{a}	2.59	3.17^{a}	2.88^{a}
1.5 (g kg^{-1} diet)	13.81^{a}	22.35^{b}	18.08^{a}	2.65	2.87^{b}	2.76^{b}
SEM[1]	0.14	0.25	0.16	0.04	0.05	0.03
p-value[2]	0.000	0.000	0.001	0.289	0.001	0.006

Different superscripts within 1 column are significantly different ($p<0.05$), [1]SEM: Standard Error Means, [2]Overall treatment p-value

(Cross *et al*., 2002; Bampidis *et al*., 2005; Krishan and Narang, 2014). Mukhtar (2011) reported that feeding broiler chicks on diet supplemented with 600 mg kg^{-1} clove oil improved body weight compared to both control and antibiotic treated groups. The aforementioned author added that the supplementation of 600 mg kg^{-1} clove oil to diets improved body weight gain compared to control group by about 2.24%. Furthermore, Lee *et al*. (2004) revealed that supplementing the essential oil to the diet of broilers improved their growth performance.

As shown in Table 3, feed intake and feed conversion ratio were significantly ($p<0.01$) affected due to the treatments except feed conversion at the first period (1-3 weeks of age). It could be noticed that feed intake increased linearly ($p<0.01$) as the level of oil mixture supplement increased. This result may be due to the enhanced palatability as a result to supplementing the diet with cold oils extracted from medicinal herbs. In agreement with the results of the present study, Mukhtar (2011) found that broiler chicks fed on diet supplemented with 600 mg kg^{-1} clove oil showed numerically increase in feed intake compared to both control and antibiotic treated groups. Mukhtar's research attributed the improvement in feed intake to the positive effects of clove oil on the digestive system and high concentration of essential oil besides that clove oil is super-rich in manganese, trace minerals which necessary for protein and carbohydrate metabolism and the synthesis of fatty acids and cholesterol. Clove oil also contains lesser amounts of omega 3 fatty acids, vitamin C and K. Results are consistent with previous reports (Ertas *et al*., 2005; Alcicek *et al*., 2003), in which different essential oils were added to poultry diets improved feed intake and feed conversion rate and carcass yield. Similar results were obtained by Abd El-Latif *et al*. (2013), who found that feed intake increased ($p<0.05$) in broiler chicken receiving diets supplemented with 100 or 200 mg rosemary oil/kg diet compared to rosemary oil free diet.

In the present study, the best feed conversion of 2.87 was associated with the treatment with 1.5 g oil mixture/kg diet at the second period (3-6 weeks of age), whilst the control group gave the best feed conversion rate within the whole experimental period (1-6 weeks of age) followed by the experimental group treated with 1.5 g oil mixture/kg diet. Similarly, Abd El-Latif *et al*. (2013) assured reported that supplementing broiler diets with rosemary oil at level of 100 or 200 mg kg^{-1} diet produced the best feed conversion ratio when compared with the control group and other treatment groups.

Cabuk *et al*. (2006) postulated that broilers fed diets supplemented with a mixture of laurel, oregano, sage, citrus and anis oils mixture significantly improved feed conversion. Essential oils favourably affect gut functions by stimulating digestive secretions such as bile and mucus and enhanced enzyme activity (Platel and Srinivasan, 2001; Manzanilla *et al*., 2004). In broilers, essential oils enhance the secretion of trypsin, amylase and jejunal chyme (Jang *et al*., 2007) and reduce the adherence of pathogens (for example, *E. coli* and *C. perfringens*) with intestinal wall (Nikaido, 1994, 2003; Jamroz *et al*., 2006).

Carcass traits: The effect of dietary supplementation with the oil mixture on carcass traits at the end of the experimental period is presented in Table 4. Results of the experiment indicated that dressing, carcass and giblets percentages were statistically ($p<0.01$) influenced by dietary treatments. The highest values of dressing and giblets percentages were observed in quails fed diets supplemented with the oil mixture at level of 0.75 g kg^{-1} diet. In the same time, feeding quails on a diet enriched with 1.5 g oil mixture/kg diet resulted in the best carcass percentage (71.03%), which increased by 3.72% comparing to those fed the control diet. In line with the present findings, Isabel and Santos (2009) found that breast weight as a percentage of carcass was significantly greater ($p \leq 0.001$) in broilers fed supplements of 100 ppm of clove and cinnamon oils than in all other treatments. Also, our results agree with observations published from previous research on essential oils (Perdok *et al*., 2003; Wald, 2003) at this facility. Mehr *et al*. (2014) found insignificant increase in carcass percentage at 42 days of age for broilers fed on diet supplemented with clove oil at level of 450 ppm/kg diet (Fig. 1).

The results of the present study disagree with those reported by Cabuk *et al*. (2006), who studied the effect of a mixture of herbal essential oils on growth and internal organ weight of broilers and concluded that there were no significant effects for the dietary treatment on body weight of the broilers at 21 and 42 days. Also, Alfaig *et al*. (2013) studied the effect of thyme essential oil on carcass traits of broiler chickens. They found that the highest carcass yield and edible parts percentage were obtained by the control group.

Impact of fixed oils on microbiological findings: At day 42, birds fed diets supplemented with increasing levels of NCR oil showed decrease ($p<0.05$) in cecum and ileum Total Bacterial

Table 4: Effect of dietary cold pressed oil supplement on carcass traits of growing japans quail at 6 week old

Items	Dressing (%)	Carcass (%)	Giblets (%)
Oils mixture effect			
0.00 (g kg^{-1} diet)	74.16^{c}	68.39^{b}	5.77^{b}
0.75 (g kg^{-1} diet)	77.31^{a}	70.99^{a}	6.32^{a}
1.5 (g kg^{-1} diet)	76.74^{b}	71.03^{a}	5.72^{b}
SEM[1]	0.49	0.44	0.10
p value[2]	0.000	0.000	0.000

Different superscripts within 1 column are significantly different ($p<0.05$), [1]SEM: Standard Error Means, [2]Overall treatment p-value

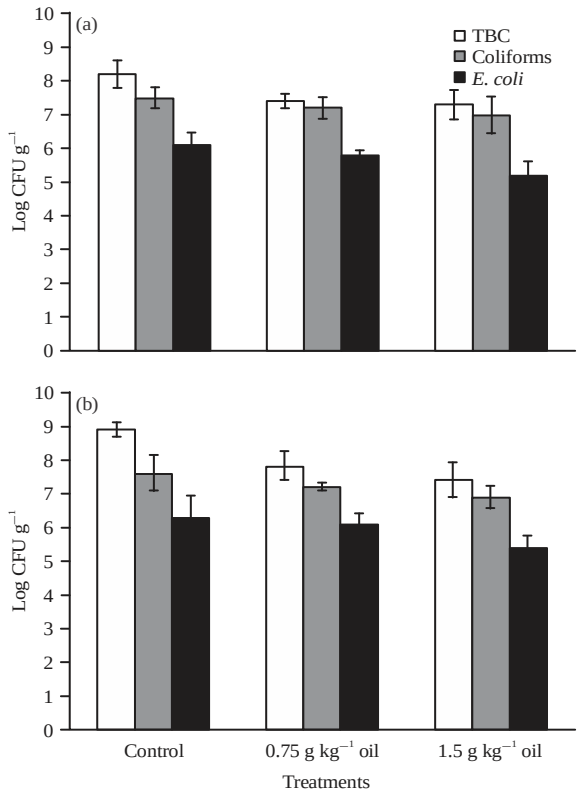

Fig. 1(a-b): Ceca and ileum microflora means (Total Bacterial Count (TBC), Coliforms, *E. coli*) of Japanese quail fed with different levels of dietary supplementation cold pressed oil mixtures after 42 days growing, (a) Ileal microflora and (b) Cecum microflora

Count (TBC), coliforms and *Escherichia coli* counts. The population of this microflora was decreased significantly ($p<0.05$) in feces after 7, 21 and 42 days comparing to those fed the control diet (data not shown). Suppression of harmful microorganisms resulted into better growth and metabolism of beneficial microbes, which might have improved the growth performance and nutrient retention in the present study. Antimicrobial activity of natural extracts and compressed oils are closely linked with their polyphenolic content (Ahn *et al*., 2004; Alzoreky and Nakahara, 2003). Therefore, plant extracts rich in phenolics and other bioactive compounds may serve as potential natural antimicrobial agents (Luther *et al*., 2007; Hyldgaard *et al*., 2012; Rahal *et al*., 2014b). These bioactive components i.e., linoleic, oleic acids, phytosterols, tocopherols and polyphenolic content in the oil mixtures (Ahn *et al*., 2004; Ramadan *et al*., 2010) suppress potentially pathogenic bacteria in the intestine and feces.

CONCLUSION

From the present findings, it could be recommended to supplement diets of growing Japanese quails with 1.5 g of the studied NCR oil mixture/kg diet to improve growth performance, carcass traits and microbiological status of both stored diet and digestive tract of birds. The NCR oil mixtures showed antimicrobial effect on the intestinal microflora and indicator of bacterial excretion in feces. The populations of TBC, coliforms and *E. coli* in the ileal and cecal contents as well as feces were fewer ($p<0.05$) in quails fed diet with NCR oil mixtures (1.5% w/w) compared with the control diet. These results indicated that dietary supplementation of antimicrobial cold pressed oils mixture (1.5 g kg^{-1} diet w/w) has the potential to enhance the growth performance and reduce intestinal and excreta pathogenic bacteria in Japanese quails.

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