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Effects of a combination of herbal oils (rosemary, black cumin, and clove) on quail growth, antioxidant enzymes and health status

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ABSTRACT

The purpose of this trial was to evaluate the potential of herbal oil combinations (rosemary, clove, and black cumin) in quail feeding as a natural growth enhancer. The effects of dietary gradual levels of this blend (RCBC) on growth indices, carcass attributes, and blood biochemical variables were compared to the control group (basal diets). For this, 300 1-week-old developing quails were employed. Birds were kept on the baseline diet with or free of herbal oils blend (RCBC) at three different amounts (0, 1.50, and 3.00 cm3/kg diet) from one to six weeks of age to suit their nutritional needs. There were no variations in live body weight or body weight gain over the entire period or at intervals. Compared to the control, birds fed RCBC-supplemented diets devoured more feed (P<0.01). RCBC supplementation in the diet did not affect the feed conversion ratio. Except for heart %, all carcass features were statistically (P <0.01) different after RCBC treatment when compared to the control. The amounts of total globulins, total protein, and albumin in quails given RCBC were higher than the control (P<0.001). In quails, the hepatic levels of GSH and the activity of SOD, catalase, GR, GPx, and GST all increased (P<0.001). MDA concentrations in hepatic homogenate were dramatically reduced by RCBC diets. Finally, RCBC supplementation at a dose of up to 3.0 cm^3/ kg diet is recommended to enhance the growth and general health of quails during growth, which would have a favorable impact on the general health of quail meat consumers.

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1 Introduction

Following the ban on antibiotics in poultry feed, natural molecules, medicinal herbs, and oilseed extracts are in high demand due to their health-promoting and biological antibacterial properties against various infections (Abd El-Hack and Alagawany 2015; Abd El-Hack et al. 2018a,b). Due to their broad beneficial benefits for immunological stimulation, growth promotion, and health care systems, herbs and their bioactive constituents are becoming important for the lifestyle of human, cattle, and poultry industries (Alagawany et al. 2015; Alagawany and Abd El-Hack 2021; Abd El-Hack and Alagawany 2022).

Because of their broad spectrum of beneficial effects on productivity, immune strengthening, and health protection, herbs, cold-pressed oils, and essential oils are becoming increasingly important in poultry production (Abd El-Hack and Alagawany 2015; Abd El-Hack et al. 2015, 2022). Furthermore, therapeutic herbs maintain the natural flora's equilibrium while also playing an important antimicrobial role against pathogens (Ghazalah and Ali 2008).

Modern commercial processes extract cold-pressed oils such as rosemary, clove oils, and black seeds by grinding the seeds of the herb under pressure. To limit the heat created by friction, the operation is carried out under thermoregulation, and the process temperature must not exceed 49°C or even be lower. The flavor, aroma, nutritional content, and medicinal characteristics of the resulting cold-pressed oils are all preserved (Lutterodt et al. 2010; Alagawany et al. 2020). To improve animal performance, phytogenic feed additives (PFA) are routinely employed. Because of their nutritional value, and improved general health and behavior of birds, they are currently regarded for poultry feeding techniques (Alagawany et al. 2015; Arif et al. 2022).

This study aimed to see how beneficial it was to use a herbal oil combination (rosemary, clove, and black cumin) in quail feeding. To do this, gradually different amounts of this mix were supplemented with a normal diet to see how they affected growth, carcass attributes, and blood biochemical traits.

2 Materials and Methods

2.1 Rations, quails, and design

A total of 1-wk-old 300 developing quails were used in this experiment with three groups (the average initial body weight was 24.25 g). Each batch was split into five replicas, each containing 20 unsexed quails. They were fed the baseline diet with or without oils blend (RCBC) from one to six weeks of age to suit their nutritional needs, according to NRC (1994). RCBC supplementation was done at three different amounts (0, 1.50, and 3.00 Cm^3/kg diet). The tested diets were as follows: 1) normal diet,

Table 1 Composition the basal diets

Ingredients	%
Corn	53.03
Soybean meal	38.69
Gluten meal	3.20
Soybean oil	1.67
Di Calcium phosphate	0.81
Vit-min Premix*	0.30
NaCl	0.11
Limestone	0.30
DL Methionine	0.39
L-Lysine HCl	1.50
Calculated analysis**:	
Crude protein %	24.04
Metabolizable energy Kcal/kg	2903
Calcium %	0.85
Available phosphorous %	0.45
Methionine + Cysteine %	0.88
Lysine %	1.60

* Growth vitamin and Mineral premix Each 2 kg consists of Vit A 12000, 000 IU; Vit D3, 2000, 000 IU; Vit. E. 10g; Vit k3 2 g; Vit B_1, 1000 mg ; Vit B_2, 49g ; Vit B_6, 105 g; Vit B_{12}, 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).

2) normal diet+1.50Cm^3 RCBC /kg diet, (3) normal diet+3.00 Cm^3 RCBC/kg diet. The feedstuffs and calculated analysis of the normal diet are shown in Table 1.

2.2 Birds, diets, and experimental design

All experimental methods were investigated according to the guidelines established by the local committee of experimental animal care and authorized by the institutional committee of ethics. The birds were kept in standard cages (50×30×50 Cm^3). All of the chicks were subjected to the same management, sanitary, and environmental circumstances. Feed and fresh water were available throughout the experiment. Throughout the trial, the birds were also kept on a 24-hour light cycle.

2.3 Data collection

Weekly, all birds were weighed to determine their LBW (body weight) and gain (BWG). The average BWG was computed by subtracting the initial LBW of a certain period from the final LBW of the same period. The author used these data to compute the average AFI (food intake) and FCR (feed conversion ratio). FCR was calculated as the number of grams of food required to produce one gram of BWG. Daily wastage of feed was recorded to estimate AFI.

Six quails (one from each group) were haphazardly selected for carcass attributes at six wks-old, weighed, and slain at the end of the trial. The liver, thigh, breast, gizzard, and heart weights were measured (g / kg slaughter wt). Dressed and carcass weights were compared to live body weight.

2.4 Blood biochemical analysis

After overnight fasting, blood samples were taken from seven quails from each group into centrifugation tubes, and the sample was centrifuged for 15 min at 5000 rpm. Serum samples were utilized to determine some biochemical parameters like ALT and AST activity using commercially available kits, as described by Breuer (1996). Protein and its fractions were analyzed using a commercial kit (Doumas et al. 1981; Drupt 1974).

The total bilirubin in the serum was determined using the available kit. The rate of production of the creatinine-picrate-colored complex influences the creatinine level in serum (Heinegard and Tiderstrom 1973). Serum lipid profiles such as total cholesterol, triacylglycerol, free fatty acids (FFA), and high-density lipoproteins (HDL) were determined by the methods of Lopes-Virella et al. (1977), Allain et al. (1974), Duncombe (1964); and Fossati and Prencipe (1982). The serum content of low-density lipoproteins (LDL) in quails was determined by the method given by Abd El-Hack et al. (2017). The oxidative status and antioxidant parameters in liver homogenate were measured by the method given by Abd El-Hack et al. (2017).

Briefly, one gram of liver tissues was weighed and homogenized with 1.17 percent chilled potassium chloride to detect the concentrations of GSH (reduced hepatic glutathione) and MDA (malondialdehyde) spectrophotometrically as described by Beutler (1969), and Nair and Turner (1984). The antioxidant enzymes in Japanese quail hepatic tissues were evaluated using Sinha (1972) and Misra and Fridovich (1972), methods. Furthermore, GST (glutathione S transferase) and GR (glutathione reductase) were found using the method of Goldberg and Spooner (1983).

2.5 Statistics

Using SAS's GLM techniques, data were treated to an ANOVA procedure with a completely randomized design (SAS Institute Inc., 2001). The author used Tukey's test to study the differences between means (P<0.05).

3 Results and Discussion

3.1 Growth measurements

Results presented in Table 2 revealed the effect of RCBC in quail diets on growth performance measures (LBW, DBWG, DFI, and

Table 2 Body weight and body weight gain of Japanese quails affected by various levels of RCBC addition

Items	LBW (g)			DBWG (g)		
	1 wk	3 wk	6 wk	1-3 wk	3-6 wk	1-6 wk
RCBC (Cm^3/kg diet)						
0.00	24.05±0.08	97.36±0.96	201.60±2.76	5.23±0.10	7.44±0.13	6.34±0.20
1.50	23.86±0.07	95.02±0.90	197.43±3.50	5.08±0.09	7.32±0.14	6.20±0.15
3.00	23.81±0.10	97.63±099	207.58±3.33	5.27±0.08	7.85±0.10	6.56±0.24
Probabilities	0.086	0.498	0.088	0.488	0.049	0.076

RCBC: herbal oils blend (equal amounts of rosemary, clove and black cumin oils); LBW: live body weight, DBWG: daily body weight gain; Means in the same column with no superscript letters after them or with a common superscript letter following them are not significantly different (P < 0.05).

Table 3 Feed intake and feed conversion ratio of Japanese quails affected by various levels of RCBC addition

Items	DFI (g/day)			FCR (g feed/ g gain)		
	1-3 wk	3-6 wk	1-6 wk	1-3 wk	3-6 wk	1-6 wk
RCBC (Cm^3/kg diet)						
0.00	13.12±0.19[b]	21.41±1.44[b]	17.26±0.50[b]	2.54±0.40	2.91±0.15	2.72±0.52
1.50	13.14±0.14[b]	23.21±1.65[a]	18.17±0.45[a]	2.62±0.38	3.29±0.16	2.95±0.10
3.00	13.94±0.26[a]	22.57±1.36[a]	18.26±48[a]	2.68±0.43	2.91±0.20	2.80±0.15
Probabilities	0.005	0.001	0.001	0.958	0.082	0.062

RCBC: herbal oils blend (equal amounts ofrosemary, clove and black cumin oils); DFI: daily feed intake, FCR: feed conversion ratio; Means in the same column with no superscript letters after them or with a common superscript letter following them are not significantly different (P < 0.05).

FCR). Further, data related to the LBW and DBWG showed no significant differences throughout the experiment and at intervals. The addition of a 3.0 Cm^3/kg RCBC diet to growing quail diets numerically raised LBW and DBWG values, albeit not significantly. This increase in LBW and DBWG could be linked to the RCBC's enhanced DFI (Table 3).

Several studies have shown that herbal oils can improve poultry growth performance by increasing nutrient digestibility, allowing them to be used as a growth enhancer (Bampidis et al. 2005). Ghazalah and Ali (2008) found that adding varying doses of rosemary leaves meal to broilers at 49 days of age raised LBW and DBWG (P<0.01). In comparison to the control, Abd El-Hack et al. (2015) pointed out that adding an oil mixture containing rosemary (1.5 g/kg diet) enhanced DBWG by 5.27 and 3.85 percent at 3-6 weeks of age and overall period (1-6-old), respectively. Alagawany and Abd El-Hack (2015) reported a non-significant influence on LBW or DBWG of the layer due to varying quantities of the rosemary plant, which is consistent with our findings. Mukhtar (2011) discovered that giving the animals a diet rich in 600 mg/kg clove oil enhanced their body weights. The addition of 600 mg clove oil /kg diet boosted BWG by 2.24 percent as compared to the control. Moreover, various active components in essential black cumin oil, including as*p-Cymence*, dithymoquinone, thymoquinone, carvacrol, and thymol may contribute to increased digestion coefficient of feed and nutrient absorption by stimulating intestinal enzymes (Salam et al. 2013). Nigellone is found in 60-80% of black seed essential oil. This chemical has been demonstrated to have antifungal and antibacterial properties that slow down the rate of growth (Shokri 2016).

The preceding data could explain why the DFI has improved. RCBC-supplemented meals resulted in higher feed consumption (P<0.01) than the control diet. The aroma of rosemary and clove extracts is pleasing, making the meal more appealing. DFI elevated (P<0.05) in a broiler diet enriched with 0.1 or 0.2 g rosemary oil/kg food relative to the control diets, according to Abd El-Latif et al. (2013). According to Mukhtar (2011) broiler chicks fed by a food supplemented with clove oil 600 mg/ kg had higher DFI than control and other treatment groups. Black cumin has also been shown to stimulate the pituitary gland, which can activate the thyroid gland directly or indirectly (Azeem et al. 2014). Thyroid hormones are necessary for body metabolism; they increase metabolic rate, which improves amino acid use by speeding up metabolism (More et al.1980).

Dietary RCBC supplementation did not affect FCR, as demonstrated in Table 3. Extracts from medicinal plants have been shown to increase the release of intestinal enzymes, improving the digestibility of certain nutrients (Ghazalah and Ali 2008). According to Nikaido (2003), essential oils can increase amylase, trypsin, and jejunal chime secretion and inhibit pathogen attachment to the intestinal wall (Jang et al. 2004).

3.2 Carcass characteristics

Except for heart percentage, all carcass features were statistically (P 0.01) different after RCBC treatment (Table 4). When compared to the control, the high dietary amount of RCBC (3 cm^3 /kg diet) produced the best results for all carcass features (dressing, giblets, liver, and gizzard). Growing a quail-fed diet supplemented with a combination of essential oils, including rosemary oil, had the best giblets and dressing percentages (Abd El-Hack et al. 2015). The results of the study suggested that supplementing the quails' diet with the mixture (1.5 g oil) per kg meal gave the highest percentage of the carcass (71.03%) when compared to the control. Furthermore, Isabel and Santos (2009) discovered that chickens fed 100 ppm cinnamon and clove oils had a higher percentage of carcass and breast (P>0.001) than all other treatment groups. In contrast, Ferket et al. (2002) investigated the effect of an essential oil blend on broiler growth and internal organ weights. The experimental treatments did not significantly affect the broiler body weights at 21 and 42 days Furthermore, Mehr et al. (2014) observed no significant change in the percentage of the carcass in broilers fed 450 ppm clove oil /kg feed.

Table 4 Carcass characteristics of Japanese quails affected by various levels of RCBC addition

Items	Carcass traits (% of slaughter weight)				
	Dressing	Giblets	Liver	Gizzard	Heart
RCBC (Cm^3/kg diet)					
0.00	74.90±0.55^{c}	5.82±0.44^{b}	2.28±0.60^{b}	2.55±0.11^{b}	0.99±0.07
1.50	77.51±0.54^{b}	5.77±0.34^{c}	2.32±0.70^{b}	2.48±0.18^{b}	0.98±0.04
3.00	78.08±0.49^{a}	6.39±56^{a}	2.50±0.69^{a}	2.94±0.15^{a}	0.94±0.10
Probabilities	0.00<	0.00<	0.00<	0.00<	0.56

RCBC: herbal oils blend (equal amounts of rosemary, clove and black cumin oils); Means in the same column with no superscript letters after them or with a common superscript letter following them are not significantly different (P < 0.05)

Table 5 The potential role of RCBC addition on some liver serum parameters, total protein and total globulins in Japanese quails

Items	TP (g/dl)	ALB (g/dl)	TG (g/dl)	ALT (U/L)	AST (U/L)	TB (mg/dl)
RCBC (Cm^3/kg diet)						
0.00	2.45±0.55^{c}	1.90±0.55^{c}	0.56±0.08^{c}	23.96±3.01^{a}	279.31±20.56	0.62±0.01
1.50	3.36±0.60^{b}	2.01±0.60^{b}	1.35±0.04^{b}	17.97±2.33^{b}	284.71±14.33	1.03±0.02
3.00	4.62±0.61^{a}	2.37±0.45^{a}	2.25±0.05^{a}	14.58±2.55^{c}	251.47±28.56	1.15±0.06
Probabilities	0.00<	0.005	0.020	0.08	0.120	0.349

RCBC: herbal oils blend (equal amounts of rosemary, clove and black cumin oils), TP: total protein, ALB: albumin, TG: total globulins, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TB: Total bilirubin; Means in the same column with different superscript letter following them are significantly different (P <0.05)

3.3 Blood biochemical parameters

When quails are given RCBC the level of total protein, albumin, and total globulins concentrations are substantially increased (P>0.001) as compared to the control group (Table 5). It's worth noting that RCBC decreased the activity of the ALT liver enzyme substantially (P=0.08). The effect of RCBC addition on liver function confirms black cumin oil's impact on enhancing liver functions and, as a result, the birds' overall health (Averbeck 1992). After feeding *Nigella sativa* to chickens, Al-Beitwai and Al-Ghousein (2008) observed similar data with the analyzed indices.

Table 6 shows the anti-atherogenic and hypolipidemic properties of RCBC. When quails were fed RCBC, blood FFA, creatinine, total cholesterol, triacylglycerol, and LDL values were lower than the control. The content of the beneficial cholesterol (HDL) was higher (P< 0.05) in birds fed diets enriched with RCBC than in control birds. These findings suggest that RCBC improved the quails' fat metabolism and liver functioning, which in turn benefited their overall health. The current findings support those of Polat et al. (2011), who discovered that rosemary oil had a hypocholesterolemic impact on chickens.

Table 6 The potential role of RCBC addition on blood creatinine and lipid profile in Japanese quails

Items	FFA (mg/dl)	CR (mg/dl)	TCH (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
RCBC (Cm^3/kg diet)						
0.00	7.44±10.01^{a}	0.81±0.05^{a}	318.57±0.10^{a}	333.58±15.23^{a}	83.75±15.23^{c}	96.46±13.54^{a}
1.50	5.40±13.23^{b}	0.56±0.06^{b}	215.84±0.11^{b}	218.49±18.21^{b}	121.71±17.56^{b}	59.59±17.25^{b}
3.00	4.02±8.65^{c}	0.45±0.09^{c}	176.08±0.03^{c}	168.10±9.89^{c}	146.73±10.67^{a}	45.70±11.34^{c}
Probabilities	0.00<	0.00<	0.00<	0.00<	0.00<	0.00<

RCBC: herbal oils blend (equal amounts ofrosemary, clove and black cumin oils), FFA: free fatty acids, CR: creatinine, TCH: total cholesterol, TC: Triacylglycerol, HDL: high density lipoprotein, LDL: low density lipoprotein; Means in the same column with different superscript letter following them are significantly different (P <0.05)

Table 7 The effect of RCBC mixture on antioxidant status in experimental groups' liver homogenates

Items	MDA (µmol/g tissue)	GSH (mg/g tissue)	CAT (µmol H_2O_2 decomposed/g tissue)	SOD (U/g tissue)	GPx (µmol NADPH/g tissue)	GR (U/g tissue)	GST (U/g tissue)
RCBC (Cm^3/kg diet)							
0.00	152.49±13.98[a]	10.51±6.55[c]	17.87±3.01[c]	8.01±7.02[c]	8.66±2.05[c]	0.79±0.57[c]	0.17±0.09[c]
1.50	87.26±9.87[b]	24.29±9.65[b]	37.28±2.88[b]	28.73±4.59[b]	17.52±1.53[b]	2.46±1.10[b]	0.45±0.10[b]
3.00	66.58±8.65[c]	30.84±6.53[a]	47.07±2.56[a]	36.45±5.96[a]	22.04±2.01[a]	3.21±0.15[a]	0.66±0.13[a]
Probabilities	0.00<	0.00<	0.00<	0.00<	0.00<	0.00<	0.00<

RCBC: herbal oils blend (equal amounts ofrosemary, clove and black cumin oils), MDA: malondialdehyde, GSH: reduced glutathione, CAT: catalase, SOD: superoxide dismutase, GPx: glutathione peroxidase, GR: glutathione reductase, GST: glutathione S transferase; Means in the same column with different superscript letter following them are significantly different (P <0.05)

Furthermore, Torki et al. (2018) discovered that the oil of rosemary lowered cholesterol and triglycerides in laying quails and had hypolipidemic effects. The main active ingredient of clove oil is eugenol, which has pharmacological properties such as anti-diabetic, and hypolipidemic properties (Khalil et al. 2017). The anti-hyperlipidemic function of eugenol was demonstrated by reduced LDL, total cholesterol, and triacylglycerol concentrations when compared to lovastatin, a lipid-lowering medicine (Venkadeswaran et al. 2014). In a hyperlipidemic zebrafish model, clove oil also lowered serum cholesterol and triacylglycerol (Jin and Cho 2011). Clove oil's hypolipidemic effect may be owing to its capacity to counteract or quench free radicals produced within the quail's body.

RCBC supplementation had a one-of-a-kind antioxidant action (Table 7). In quails, the levels of liver GSH and the activity of GPx, SOD, catalase, GR, and GST all increased (P>0.001) (Table 7). The MDA in hepatic homogenate receiving RCBC diets, on the other hand, was substantially lower than the control. These findings agree with those of Cetin et al. (2017) who discovered that the oil of rosemary improved markers of oxidative stress in quails and hens, respectively. Bulbul (2012) also found that supplementing rosemary and oregano oils together has anti-oxidant effects in quails.

The antioxidant effect of clove oils may be attributed to eugenol, which has an allyl group in its structure and creates an iron-oxygen chelate complex (Ito et al. 2005). Clove oil helps stop hydroxyl radicals from forming, which are subsequent results of lipid peroxidation (Ito et al., 2005). According to Halc et al. (2012), the oil of black cumin improves the antioxidant system and reduces MDA, DNA, and protein damage, all of which help the defense of birds against oxidative damage and affect general health and overall performance.

Conclusions

The current findings demonstrated that feeding developing Japanese quail with a diet supplemented by RCBC boosted the amount of feed intake, BWG, and carcass features. Furthermore, adding 1.5 and 3.0 Cm^3 RCBC/kg to the diet enhanced liver function by lowering serum creatinine, total cholesterol, LDL, triacylglycerol, and FFA while increasing HDL concentration. RCBC supplementation had anti-oxidant benefits, raising GSH levels and SOD, GR, GPx, and GST activities while decreasing MDA concentration. RCBC supplementation at a dose of up to 3.0 Cm3/ kg diet is indicated to promote the growth performances and health of quails during growth.

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Conflict of interest

None

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