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Original Research

The use of a mixture of essential oils in meat broiler diets and its effect on physiological and textile characteristics

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ABSTRACT:

This study was conducted in the poultry Farm of Animal production Department, College of Agriculture, University of Baghdad, the previous site in Abu Ghraib for the period from 1.10.2017 to 11.11.2017. This study demonstrates the effect of using a mixture of essential oils in broiler diets and some physiological and histological traits. A total of 375 unsexed Ross 308 one day old use was in this experiment. Birds were distributed randomly on five treatment and three replicates per treatment, each replicates containing 25 chicks (75 chick/treatment). Treatments were as follows: Treatment (T_1) control group (without any supplement of oil mixture (essential oils) and T_2, T_3, T_4 and T_5 included the addition of (black seed oil, cumin oil, parsley oil, anise oil and grape seed oil) oil mixture (0.25, 0.50, 0.75 and 1%) respectively. The results showed there were no significant difference in the concentration of glucose and the value of peroxide, while the superiority of the total protein for the public of treatment T_2 and the superiority of the cholesterol ratio for the treatment of the fifth T_5 was also observed to have a significant superiority in favor of treatment T_3 in T_4 Triglycerides and HDL while T_5 was superior to LDL and T_1 was treated in VLDL ratio. And a significant superiority ($P<0.05$) in the villi length and crypt depth were seen for the ileum and jejunum partial.

Keywords:

Partial substitution, Mixture of aromatic oils, Villi length, Crypt depth.

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INTRODUCTION

The use of preventive doses of antibiotics in poultry feeding has improved the growth and consumption of fodder, reduced pathogens and reduced related diseases. However, the growing concern about the spread of antimicrobial resistance and the accumulation of these antibiotics in animal products has prompted organizations to ban them within the EU 2006 (Zeng *et al*., 2015). Therefore, it is necessary to find additions from natural sources as effective alternatives to improve the overall performance of poultry and, more importantly, they have less serious side effects on human health, so new commercial additions derived from plants and aromatic plant extracts and its purification component as a part of the alternative food strategy for the future. Essential oils contain many different antimicrobial compounds, such as hydrocarbons, phenols, ketones, esters and ethers (Solórzano-Santos *et al*., 2012; Marchese *et al*., 2017).

These oils have several properties that make them a future alternative to antibiotics because of their complex and varied effects due to the various chemical components and different therapeutic methods. Their role in activating the growth efficiency and its positive effect on the digestive system by stimulating the juices and digestive enzymes such as amylase and lipase. (Placha *et al*., 2014; Pirgozliev *et al*., 2015; Yang *et al.*, 2018). In the last years researchers have been interested in studying the role of mixtures of essential oils as feed additives in poultry diets, as most studies around the world are based on the possibility of merging and making different combinations of essential oils or extracts of active substances based on their active principles and adoption as a natural antibiotic (Gopi *et al*., 2014). The use of essential oils in poultry diets have led to the enhancement of the digestive qualities and increase the secretion of the digestive system and stimulate the circulation of the blood and to give the biological properties of antioxidants and reduce the levels of bacteria causing diseases as well as the potential to strengthen the immune system (Brenes and Roura, 2010).

MATERIALS AND METHODS

The experiment was conducted in the poultry field of the Department of Animal Production, Faculty of Agriculture, University of Baghdad, Abu Ghraib Old site for the period from 1.10.2017 to 11.11.2017 and for a period of 42 days to study the effect of adding different levels of a mixture of some essential oils replacing the yellow corn oil in broiler diets in some histological and physiological characteristics. This study used (375 nos) unsexed broiler chicks (308 Ross) strain one day-old and weight at a rate of 37.37 g/chick. Fed chicks on diet (starter) of age (1 - 10 days), then growth diet (growth) of age (11-24 days) and the third is the final of the diet (finisher) of age (25-42). According to the Ross 308 manual as shown in Table 1. Water and feed were provided free of charge during the trial period. A combination of the above oils was prepared by mixing 20% of each oil to have a 100% portion of this synthesis. The experiment coefficients were distributed as follows: Treatment (T_1) control group (without any supplement of oil mixture (essential oils) and T_2, T_3, T_4 and T_5 included the addition of (black seed oil, cumin oil, parsley oil, anise oil and grape seed oil) oil mixture (0.25, 0.50, 0.75 and 1%) respectively.

The study of some physiological characteristics after random blood collection of four birds per duplicate, with 12 birds per treatment at the end of the experiment at the age of 42 days. The blood was then discharged directly into 6 mL glass tubes containing gel tubes. These tubes were placed in the centrifuge at 4000 cycles/minute and for 10 minutes to separate the serum from the cellular part and kept at a temperature of -20°C until the tests. And then conducted the following tests for serum [measuring total protein concentration (Wootton *et al*., 1982) the concentration of glucose (Kaplan and Glucose, 1984), cholesterol concentration

Table 1. Percentage of ingredients used in the study and their chemical composition

S. No	Feeding material	The beginning of the food (1-10 days)	Fruity growth (11-24 days)	Ultimate feed (25-42 days)
1	Yellow corn	47.7	61	63
2	Grilled wheat (local)	10	-	-
3	Soybeans 48% protein	33	29.4	26.35
4	Proteins [(1)]	5	5	5
5	Yellow corn oil	2	2.6	3.8
6	limestone	1.1	1.1	1.1
7	DCP	0.7	0.5	0.35
8	Food salt	0.3	0.2	0.2
9	Mixes of vitamins and minerals [(2)]	0.2	0.2	0.2
	Total	100	100	100
	Calculated chemical analysis [(3)]			
10	Raw protein (%)	23	21.3	20
11	Representative energy calculated (kcal/kg feed)	3000.5	3100.2	3200.7
12	Lysine (%)	1.3	1.2	1.1
13	Methionine (%)	0.50	.049	0.47
14	Cysteine (%)	0.37	0.34	0.32
15	Methionine + Cysteine (%)	0.87	0.796	0.74
16	Argene (%)	1.36	1.25	1.1
17	Calcium (%)	0.92	0.87	0.82
18	Available phosphorus (%)	.047	0.43	0.40

1. Proteins Status BROCON - 5 SPECIAL W. Each containing 40% raw protein, 5% fat, 2.26% fiber, 5% calcium, 4.68% phosphorus, 3.85% lysine, 3.7% methionine, 4.12% methionine + cystine, 2.4% sodium, 2107 kg / kg , Vitamin B, 20 mg Folic acid, 100 mg Biotin, 100 mg Vitamin B, 140 mg Vitamin B2, 80 mg IU, 600,000 IU Vitamin D, 600 mg Vitamin E, 1 mg iron, 200 mg copper, 1.6 mg manganese, 1.2 mg zinc, 20 mg iodine, 5 mg selenium, 900 mg antioxidant (BHT).
2. A mixture of vitamins and minerals Each kg contains: 500 IU Vitamin A, 600 IU D3, 10 mg E, 2 mg K3, 2 mg B1, 2 mg B2, 2 mg B6, 5 microgram B12, 10 mg C, 15 mg niacin, 500 µg folic acid.
3. According to the chemical analysis of the suit according to NRC (1994).

(Kaplan and Glucose, 1984), triglycerides concentration (Bucolo and David, 1973), the concentration of High Density Lipoproteins (HDL) (Kaplan and Glucose, 1984), the concentration of Low Density Lipoproteins (LDL), the concentration of Very Low Density Lipoproteins (VLDL) (Friedewald *et al*., 1972) and peroxide value (Beckman *et al*., 1992)]. The histological study was carried out after slaughtering the birds. The internal intestines were extracted and the intestines were separated. Three sections of each bird were taken from the first duodenum, the second from the ileum and the third from the jejunum, 4 birds/treatment, and the length of the sample does not exceed 2 cm for the purpose of conducting a histological study. Samples were kept in plastic containers containing formalin, which was prepared at a concentration of 10% to protect them from degradation. Histological segments were introduced according to method (Bancroft and Stevens, 2010). Use the full random design CRD (Completely Randomized Design) in the study of the effect of various transactions in the studied traits, and compared to the moral differences between the averages test (Duncan, 1955) border and ready-use program SAS (2012) in the statistical analysis.

RESULTS AND DISCUSSION

The effect of adding a mixture of essential oils at different rates to the diet in the glucose concentration

Table 2. Effect of adding a mixture of aromatic oils with different concentrations to the diet in the blood biochemical characteristics (average ± standard error) of the broiler diet at 42 days

S. No	Treatment	Glucose (mg/1000mL serum)	Total protein (g/100 mL serum)	Cholesterol (mg/mL serum)	Triglycerides (mg/mL)	HDL (Mg/100 mL serum)	LDL (Mg/100 mL serum)	VLDL (mg / 100 mL serum)	Proxy (µm / 10 mL serum)
1	T_1	195.50±14.50	3.55±0.150	202.50±2.50^{AB}	108.50±3.50^{C}	31±1.0^{C}	150±1.0^{AB}	36.50±1.5^{A}	0.135±0.015
2	T_2	191.00±9.0	4.30±0.10	192.50±8.50^{B}	155.00±13.0^{B}	36±1.0^{B}	126±7.0^{BC}	30.50±2.50^{B}	0.125±0.015
3	T_3	205.00±0.0	3.95±0.050^{B}	199.50±4.50^{AB}	184.00±6.0^{A}	40±1.0^{A}	123±4.0^{C}	21.50±0.50^{C}	0.160±0.0
4	T_4	201.00±12.0	4.25±0.050^{AB}	188.00±6.0^{B}	140.50±4.50^{B}	35±1.0^{B}	125±8.0^{C}	28.00±1.0^{B}	0.155±0.015
5	T_5	199.50±15.50	3.95±0.050^{B}	218.00±8.0^{A}	107.50±2.50^{C}	31±1.0^{C}	165.50±9.50^{A}	21.50±0.50^{C}	0.115±0.015
6	Moral level	N.S	** 0.01	* 0.05	** 0.01	** 0.01	* 0.05	** 0.01	N.S

The averages that carry the different letters within the same column are significantly different (P <0.05) or (P <0.01) among them.
Treatment: T_1: Control treatment 0% Aromatic oils mixture; T_2: Treatment Add aromatic oils mixture by 0. 25%; T_3: Treatment of adding essential oils mixture by 0.50%; T_4: Treatment of adding aromatic oils mixture by 0.75%; T_5;: Treatment of adding essential oils mixture By 1%.

of the blood serum are shown in Table 2. There were no significant differences between all the experimental parameters, although there was a decrease in the benefit of the second treatment T_2, which amounted to 191 mg/100 mL serum compared to the other treatments.

Table 2 shows the effect of adding a mixture of essential oils at different rates to the diet in the total protein concentration of the serum. The results showed that there were significant differences (P<0.01) for all the mixture coefficients on the treatment of the comparison. The advantage was for the second treatment T_2 (adding the mixture of essential oils by 0.25%) which recorded 4.30 g/100 mL plasma blood, compared with T_1, T_3 and T_5 (3.55, 3.95 and 3.95 g/100 mL) blood plasma respectively. There were no significant differences with T_4, which was 4.25 g/100 mL plasma. The results differed with a study carried out by (Jaffer, 2017) in which grape seed oil was used (10, 25, 50 and 75%) in pigeons diet. The results showed no significant differences in total protein concentration between all treatments.

Table 2 shows the effect of adding a mixture of essential oils at different rates to the diet in the total cholesterol concentration in the serum. A significant decrease (P>0.05) was observed for both treatments T_2 and T_4, which recorded 188 (192.5 mg/100 mL serum) respectively, compared with other experimental parameters. Craig (1999) suggests that medicinal herbs and essential oils have cholesterol-lowering properties by inhibiting the action of a 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) in the liver. Table 2 shows the effect of adding a mixture of essential oils at different rates to the diet in the concentration of the triglycerides. The results showed that there were significant differences (P<0.01) for the third treatment T_3 (adding 0.5% of aromatic oils), which recorded (184) mg/100 mL serum compared to the rest of the treatments, followed by the second treatments T_2 and the fourth T_4, which recorded 140.50 mg/100 mL serum respectively

Table 3. Effect of the addition of a mixture of essential oils in different concentrations to the diet in the histological characteristics of the duodenum, ileum and jejunum in the villa length and crypt depth (average ± standard error) (µm) of the broilers at 42 days

S. No	Treatment	Duodenum		Ileum		Jejunum	
		Vila length (µm)	Crypt depth (µm)	Villa length (µm)	Crypt depth (µm)	Villa length (µm)	Crypt depth (µm)
1	T_1	103.33±8.81	15.33±2.90	108.33±4.40^{AB}	16.33±1.85^{B}	49±3.51^{BC}	14.33±1.45^{AB}
2	T_2	98.33±1.66	12.66±1.45	91.66±8.33^{B}	10.66±0.66^{B}	43.66±0.88^{C}	10.33±0.88^{B}
3	T_3	110±10.0	19.33±4.33	100.33±0.33^{AB}	15.33±0.88^{B}	53.33±4.70B^{C}	10.33±1.85^{B}
4	T_4	106.67±12.01	11.33±1.33	116.66±8.81^{A}	23.33±3.33^{A}	58.66±2.90^{B}	12±1.52^{B}
5	T_5	113.33±8.81	11.66±0.88	98.33±4.40^{AB}	12±1.52^{B}	77.33±3.71^{A}	17.66±1.20^{A}
	Moral Level	N.S	N.S	* 0.05	** 0.01	** 0.01	* 0.05

- The averages that carry the different letters within the same column are significantly different (P<0.05) or (P<0.01) among them.
- Treatment: T_1: Control treatment 0% Aromatic oils mixture; T_2: Treatment Add aromatic oils mixture by 0.25%; T_3: Treatment of adding essential oils mixture by 0.50%; T_4: Treatment of adding aromatic oils mixture by 0.75%; T_5: Treatment of adding essential oils mixture by 1%.

compared with T_1 control which amounted to 108.50 mg/100 mL serum and an improvement was observed for the fifth treatment T_5, with the concentration of triglycerides decreased by 107.50 mg/100 mL. The results differed with a study conducted by (Mashhadani and Alaradi, 2014). Cumin seeds were used in percentages (0.5, 1 and 1.5 g/kg feed) and cumin oil at (250, 500 and 750 mg/kg feed). There were no significant differences between the treatments in the concentration of triglycerides.

Table 2 shows the effect of adding a mixture of aromatic oils in different proportions to the diet in fatty proteins concentration of high-density HDL serum, of noted the presence of high moral (P<0.01) for the third treatment T_3 addition essential oils mixture ratios (0.50%) recorded (40 mg/100 mL) compared with the rest of the transactions, followed by two treatments second T_2 fourth T_4, amounting to 36 and 35 mg/100 mL respectively, compared with T_1 (31 mg/100 mL) and T_5 (31 mg/100 mL), respectively. These results were agreed with the results of Hong *et al*. (2012) when adding a mixture of essential oils consisting of marjoram, anise and citrus peel by 125 ppm for the meat breeds, noting that a significant increase (P<0.05) for the treatment of the addition of essential oils.

The data in Table 2 show that the effect of adding a mixture of essential oils at different rates to the diet in the concentration of Low Density Lipoproteins (LDL) of the serum showed a significant decrease (P<0.05) for T_3 and T_4, which recorded 123 and 125 mg/100 mL respectively, followed by T_2 (126 mg/100 mL serum) compared to the first T_1 treatment (150 mg/100 mL serum in LDL concentration). These results differed with those of Hong *et al*. (2012) when adding a mixture of essential oils consisting of marjoram, anise and citrus peel by 125 ppm for meat breeds, with no significant differences.

The results in Table 2 indicated the effect of adding a mixture of essential oils at different rates to the diet in the concentration of VLDL of the serum to a high (P>0.01) T_1 control (without adding the essential oils mixture) which amounted to 0.50 mg/100 mL compared to T_2, T_3, T_4 and T_5 (0.50, 0.75 and 1%) respectively, which was 30.50, 21.50, 28 and 21.50 mg/100 mL serum respectively, as the same table explains a significant decrease in favor treatment of aromatic oils. These results were agreed with the results obtained by Hong *et al*. (2012) when adding a mixture of essential

oils consisting of marjoram, anise and citrus peel by 125 ppm for the meat breeds. There were significant differences in the treatment of the addition of soft oils compared to the treatment of antibiotics and control.

The data in Table 2 indicated that there is no significant difference in the value of peroxide among all experimental factors, but superiority in favor of the third and fourth treatment (adding the mixture of essential oils by 0.50 and 0.75%, respectively) on the rest of the experiment transactions. The results, which included some of the physiological characteristics studied in the experiment, can be said that the treatments of adding the mixture of aromatic oils gave positive results. This improvement may be due to the containment of the mixture of essential oils on active compounds if these substances stimulate the secretion of steroid hormones (Liu *et al*., 2005), which increase metabolism as they act as building hormones that help to form proteins and reduce their degradation (Kumagi *et al*., 1957), perhaps the improvement was due to a combination of active compounds. Badary *et al*. (2000) confirmed that the role of thymoquinone in black seed oil reduced serum triglyceride levels. The improvement in the concentration of HDL may be due to the fact that plant essential oils contain a high level of unsaturated fatty acids that increase the concentration of HDL.

Shinagawa *et al*. (2015) noted that grape seed oil contains a high percentage of unsaturated fatty acids (up to 85% -90%), including linolenic acid and oleic acid. Unsaturated fatty acids have an important role in oxidative balance and regulation of promoting inflammatory and immune response (Raphael and Sordillo, 2013). The combination of essential oils contains active compounds, including flavonoids, which have the ability to inhibit free radical activity because they have an effective antioxidant role within the body. They provide protection for cholesterol, low-density lipoprotein, of oxidation and elimination of harmful LDL molecules that cause clogged blood-vessel (Arora *et al*., 2000). These results differed with that obtained by (Hong *et al*., 2012) when adding a mixture of aromatic oils composed of (marjoram and anise and citrus peel) by 125 ppm on a broiler, as there were no significant differences. This increase may be due to the fact that the mixture of essential oils and its active compounds has a high capacity as an effective antioxidant (Burt, 2004). Especially vitamin E, which has a vital role in protecting unsaturated fatty acids and other cell membrane components (Lanari *et al*., 2004).

The data in Table 3 indicated the effect of adding a mixture of essential oils at different rates to the broiler in the length of the villi length and crypt depth (morphological characteristics of the small intestine) of the histological section of the (duodenum, ileum and jejunum) showed no significant differences ($P<0.05$) in the villi length and crypt depth of the histological sections of the duodenum for all treatments. As for the ileum part, the results showed significant differences ($P<0.05$) in the villi length for treatment (T_4) (add a mixture of essential oils 0.75%) compared with the second treatment T_2 (add a mixture of essential oils 0.25%) and the presence of a highly significant difference ($P>0.01$) in depth crypts of ileum for the treatment of the fourth T_4 (add aromatic oils mixture 0.75%), which reached the length of villus where 23.33 µm compared with the rest of the experiment coefficients (T_1, T_2, T_3 and T_5) which recorded (16.33, 10.66, 15.33 and 12 µm) respectively. For the part of the jejunum, the data in Table 3 indicated a highly significant superiority ($P<0.01$) in the length of the villi to treatment of the T_5 compared with the all treatment as it recorded 77.33 µm, followed by the T_4, which recorded 58.66 µm compared to the T_1, which was 49 µm, and the significantly ($P<0.05$) in the depth of crypts for the part of jejunum to T_5 which recorded 17.66 µm compared to control T_1 which was 14.33 µm.

The improvement in the length of the villi and the depth of the crypt for the ileum and jejunum parts in

the small intestine. This improvement may be due to the effect of adding a mixture of essential oils to the diet, which in turn led to an improvement in digestion processes and thus increased absorption of the digestive system, which reflected positively on body weight and increases the feed consumption. These essential oils also stimulate the digestive system and improve the digestibility of nutrients such as proteins, fats and complex carbohydrates. Therefore, the mixture of essential oils improves the digestibility of the virtual digestion and this is reflected positively on the performance of birds. The presence of some compounds such as cuminaldehyde, thymoquinone, Linalool, geranio in the mixture of essential oils which act as antibacterial agents against harmful microorganisms as they work to stabilize microbial for beneficial microorganisms and improve gastrointestinal environment. This encourages the growth of microorganisms beneficial and therefore works to increase the utilization of food through enzymes produced by these useful neighbourhoods, which is reflected positively on the performance of birds. These compounds also play a catalytic role for some enzymes such as trypsin, amylase, lipase, and therefore provide a good opportunity for digestion and absorption of nutrients, which is reflected positively on body weight and thus increase weight (Shabaan *et al*., 2012). These results differed with those obtained by (Hong *et al*., 2012) when adding a mixture of essential oils consisting of marjoram, anise and citrus peel by 125 ppm for broiler diet. There were significant differences in the treatment of the addition of essential oils compared with the treatment of antibiotics and control in the villi length and crypt depth for the duodenum and there were no difference in the length and depth of the villi and crypt in the ileum and jejunum parts. These results were consistent with a study conducted by Al-Mashhadani and Al-Aradi (2014). Cumin seeds were used by them in the concentration of (0.5, 1 and 1.5 g/kg) feed and cumin oil at (250, 500 and 750 mg/kg) feed. This study showed significant improvement In the length of the villi and the depth of the crypt for the ileum part.

CONCLUSION

The use of supplementary feeding for a mixture of aromatic oils included black seed oil, cumin oil, parsley oil, anise oil and grape seed oil at 0.25, 0.50, 0.75 and 1% respectively in broiler diet significantly improved the physiological traits superior to total protein and HDL, lowering both the level of glucose, cholesterol level, and VLDL in the blood. Essential oil mixture could be considered as a potential growth promoter for poultry diet and can be used as an alternative to an antibiotic.

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