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The effect of thymol and carvacol oils supplementation on meat quality of broiler chickens

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A B S T R A C T

This study evaluates the effect of thymol and carvacrol supplementation on meat chemical and microbiological quality of broiler chicken. 120 one day old chicks are purchased and divided into 4 groups treated with different concentrations (4g, 5g, and 6g) of thymol and carvacrol (1:1) for each 20 kg of feed with a control group which has no thymol and carvacrol. At a 42nd day old, 8 birds collected randomly from each group and cut to breast and thigh muscles for examination their quality chemical and microbiological examinations. The results revealed that total volatile nitrogen (TVN) decreased from (9.07 ± 0.31) mg% in control group till (3.55 ± 0.18) mg% in group C with the highest thymol and carvacrol containing meals in thigh muscle samples. Also, thiobarbituric acid (TBA) decreased from (0.26 ± 0.02)mg/kg in control group to (0.12 ± 0.01)mg/kg in group C with the highest thymol and carvacrol concentration in meals in thigh muscle samples. Also, total aerobic bacterial count decreased from ($9.13\times10^4 \pm 2.01\times10^4$) cfu/g in control group to ($5.02\times10^3 \pm 0.96\times10^3$) cfu/g in C group (6 g of thymol and carvacrol)in thigh muscle samples, total coliforms decreased from ($1.17\times10^4 \pm 0.20\times10^4$) cfu/g in control group to ($1.75\times10^3 \pm 0.27\times10^3$) cfu/g in group C , total staphylococcus count also reduced from ($7.17\times10^3 \pm 1.63\times10^3$) cfu/g in the control group to ($9.18\times10^2 \pm 2.01\times10^2$) cfu/g in group C (6 g)Total fungal count reduced from ($1.56\times10^3 \pm 0.21\times10^3$) cfu/g to ($5.31\times10^2 \pm 0.82\times10^2$) cfu/g in group C. The results revealed the antioxidant and antimicrobial effect of thymol and carvacrol. Also, thymol and carvacrol improved meat quality in the samples.

Keywords: Poultry meat; Thymol and Carvacrol; Meat quality

1. Introduction

Poultry meat has great interest over the world. Quality of this meat is the most important factor for the consumer. Lipid oxidation is one of the primary mechanisms of quality deterioration in foods, especially in meat and meat products (Morrissey et al., 1998).

There is a new trend for preservatives to replace synthetic preservatives by natural ones. So, scientists searched natural substances for their capability of preservation as Laurel leaves that have provided additional protection of meat against microbial growth and increased its shelf life (Saleh., 2018).

Supplementation also is a good way to improve meat quality. To maximize the oxidative stability of meat, antioxidants, mostly α-tocopherol acetate (ATA), are added to feeds. The beneficial effect of dietary ATA supplementation for the subsequent enhanced stability of lipids in muscle foods has been extensively reported for poultry, beef cattle, veal calves, and pigs (Jensen et al., 1998).

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In poultry production, owners use synthetic antioxidants as butylated hydroxianisole as antioxidants (Chastain et al., 1982). Scientists examine new natural additives as antioxidants to minimize the use of synthetic ones because of their suspected carcinogenic effect (Chen et al., 1992) considering that administration of natural antioxidants in diets caused oxidative stability of meat or meat products (Tang et al., 2000).

Oregano essential oils have antifungal (Dauk et al., 1995), antioxidant (Yanishlieva et al., 1999; Cervato et al., 2000), and antimicrobial activities (Dorman and Deans, 2000). Thymol and carvacrol which are the main components of oregano are responsible for those properties (Lambert et al., 2001).

So, the aim of this study to examine the possible use of thymol and carvacrol as natural feed additives to improve broiler meat quality by investigating their antioxidant and antimicrobial properties and usage of thymol and carvacrol as natural antioxidants instead of synthetic antioxidants because of their suspected carcinogenic effect.

2. Material and methods

3.1. Collection of samples

120 one day old chicks are purchased and divided into 4 groups treated with different concentrations of thymol and carvacrol on the food. Each group contains 30 chicks. Group A is supplied with 4 g of thymol and carvacrol (1:1) for each 20 Kg of feed, Group B is supplied with 5 g of thymol and carvacrol(1:1) for each 20 Kg of feed, Group C is supplied with 6 g of thymol and carvacrol(1:1) for each 20 Kg of feed, and the control one with no thymol and carvacrol on the feed.

At a 42nd day, 8 chicks are collected randomly from each group, slaughtered by neck dislocation, eviscerated, packed then sent to lab for examination and evaluation of meat quality of each group. Each chick divided into thigh muscle sample and breast muscle (ISO 6887: 2003).

3.2. Sensory evaluation (World's Poultry Science Association., 1987)

3.3. Chemical examination

3.3.1. Determination of pH value according to (ISO 2917:1999)

3.3.2. Determination of Total Volatile Nitrogen (TVN) according to (EOS: 63-9/ 2006)

3.3.3. Determination of Thiobarbituric Acid Number (TBA) according to (EOS: 63-10/2006)

٣.4. Microbiological examination

3.4.1. Preparation of samples (ISO 6887-2: 2003)

To 10 grams of the sample, 90 ml of sterile peptone water were added and thoroughly mixed using a sterile blender for only 1 minute to avoid increasing its temperature, from which tenfold serial dilutions were prepared. The prepared samples were subjected to the following examinations:

3.4.2. Total Aerobic Bacterial Count "TABC" according to (ISO 4833:2003)

3.4.3. Total Coliform count according to (ISO: 4832:2006)

3.4.4. Staphylococcus count according to (ISO 6888-1:2003)

3.4.5. Total fungal count according to (ISO 21527:2001)

3.5.6. Statistical Analysis

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to (Feldman et al., 2003).

Table 1: Influence of supplementation of thymol and carvacrol on sensory characteristics of the examined samples of chicken breast.

Character / Groups	External aspect (3)	Odor (3)	Color (3)	Muscular elasticity (3)	Overall Score (12)	Sensorial Quality
Control	2	2	3	2	9	Excellent
A	3	2	3	3	11	Excellent
B	2	2	3	3	10	Excellent
C	3	2	3	2	10	Excellent

A= Chicken fed on ration supplemented with 4 g thymol & carvacrol/ 20 Kg of the feed. B= Chicken fed on ration supplemented with 5 g thymol & carvacrol/ 20 Kg of the feed.

C= Chicken fed on ration supplemented with 6 g thymol & carvacrol/ 20 Kg of the feed.

Table 2: Influence of supplementation of thymol and carvacrol on sensory characteristics of the examined samples of chicken thigh.

Character / Groups	External aspect (3)	Odor (3)	Color (3)	Muscular elasticity (3)	Overall Score (12)	Sensorial Quality
Control	3	2	3	2	10	Excellent
A	3	2	3	3	11	Excellent
B	3	2	3	3	11	Excellent
C	2	2	3	3	10	Excellent

Table 3: Influence of feeding on pH values in the examined samples of chicken meat

Tissues / Groups	Thigh			Breast		
	Min	**Max**	**Mean ± S.E***	**Min**	**Max**	**Mean ± S.E***
Control	5.78	5.85	5.81 ± 0.01	5.83	5.92	5.87 ± 0.01
A	5.74	5.79	5.76 ± 0.01	5.75	5.83	5.79 ± 0.01
B	5.69	5.77	5.72 ± 0.01	5.71	5.78	5.74 ± 0.02
C	5.60	5.68	5.64 ± 0.01	5.65	5.73	5.68 ± 0.01

S.E* = standard error of mean PH mean value for group A, B, and C was (5.76 ± 0.01, 5.72 ± 0.01 and 5.72 ± 0.01) receptively and for control was (5.81 ± 0.01) in thigh muscle samples.

Table 4: Analysis of variance (ANOVA) of pH values in the examined chicken meat samples

Source of variance	D.F	S.S	M.S	F.value
Total	63	3.3012		
Between Feeding (F)	3	0.7369	0.2457	5.92 ++
Between Tissues (T)	1	0.1398	0.1398	3.37 +
(F) × (T) interaction	3	0.1004	0.0336	0.81 NS
Error	56	2.3241	0.0415	

D.F = Degrees of freedom ++ = High significant differences (P<0.01) S.S = Sum squares NS = Non significant differences M.S = Mean squares

Table 5: Influence of supplementation of thymol and carvacrol on TVN-B values (mg %) in the examined samples of chicken meat

Tissues / Groups	Thigh			Breast		
	Min	**Max**	**Mean ± S.E***	**Min**	**Max**	**Mean ± S.E***
Control	6.98	11.24	9.07 ± 0.31	5.79	9.13	7.84 ± 0.21
A	4.17	8.62	6.43 ± 0.25	3.65	6.88	5.10 ± 0.16
B	3.35	7.09	5.19 ± 0.23	2.58	4.91	3.96 ± 0.14
C	2.41	5.87	3.55 ± 0.18	1.92	4.06	2.71 ± 0.09

S.E* = standard error TVN mean values for group A, B, and C for thigh muscle samples were (6.43 ± 0.25, 5.19 ± 0.23and 3.55 ± 0.18) mg% receptively and for control was (9.07 ± 0.31) mg% in thigh muscle

Table 6: Analysis of variance (ANOVA) of TVN-B values in the examined chicken meat samples

Source of variance	D.F	S.S	M.S	F.value
Total	63	82.7362		
Between Feeding (F)	3	24.5018	8.1672	9.15 ++
Between Tissues (T)	1	5.6412	3.5763	6.32 ++
(F) × (T) interaction	3	2.6085	0.8679	1.04 NS
Error	56	49.9847	0.8926	

D.F = Degrees of freedom ++ = High significant differences (P<0.01) S.S = Sum squares NS = Non significant differences M.S = Mean squares

Table 7: Influence of supplementation of thymol and carvacrol on TBA values (mg/Kg) in the examined samples of chicken meat

Tissues	Thigh			Breast		
Groups	**Min**	**Max**	**Mean ± S.E***	**Min**	**Max**	**Mean ± S.E***
Control	0.20	0.33	0.26 ± 0.02	0.15	0.27	0.21 ± 0.02
A	0.14	0.25	0.19 ± 0.02	0.13	0.20	0.16 ± 0.01
B	0.11	0.21	0.15 ± 0.01	0.07	0.15	0.11 ± 0.01
C	0.09	0.14	0.12 ± 0.01	0.06	0.11	0.08 ± 0.01

S.E* = standard error M.S = Mean squares Tba mean values for group A, B, and C for thigh muscle samples were (0.19 ± 0.02, 0.15 ± 0.01, and 0.12 ± 0.01) mg/kg receptively and for control was (0.26 ± 0.02) mg/kg.

Table 8: Analysis of variance (ANOVA) of TBA values in the examined chicken meat samples

Source of variance	D.F	S.S	M.S	F.value
Total	63	2.0766		
Between Feeding (F)	3	0.3315	0.1106	4.01 +
Between Tissues (T)	1	0.0897	0.0897	3.25 +
(F) × (T) interaction	3	0.0930	0.0310	1.12 NS
Error	56	1.5624	0.0276	

D.F = Degrees of freedom + = Significant differences (P<0.05) S.S = Sum squares NS = Non significant differences M.S = Mean squares

Table 9: Influence supplementation of thymol and carvacrol on Total Aerobic Plate Counts "TABC" (cfu/g) in the examined samples of chicken meat

Tissues	Thigh			Breast		
Groups	**Min**	**Max**	**Mean ± S.E***	**Min**	**Max**	**Mean ± S.E***
Control	3.9×10^4	5.4×10^5	$9.13\times10^4 \pm 2.01\times10^4$	1.7×10^4	2.6×10^5	$7.48\times10^4 \pm 1.43\times10^4$
A	7.6×10^3	1.0×10^5	$2.47\times10^4 \pm 0.35\times10^4$	6.0×10^3	8.3×10^4	$1.15\times10^4 \pm 0.21\times10^4$
B	2.8×10^3	4.1×10^4	$6.81\times10^3 \pm 1.12\times10^3$	2.2×10^3	1.9×10^4	$3.93\times10^3 \pm 0.58\times10^3$
C	2.3×10^3	1.2×10^4	$5.02\times10^3 \pm 0.96\times10^3$	1.0×10^3	7.5×10^3	$2.20\times10^3 \pm 0.29\times10^3$

S.E* = standard error of mean TABC mean values for group A, B, and C for thigh muscle samples were ($2.47\times10^4 \pm 0.35\times10^4$, $6.81\times10^3 \pm 1.12\times10^3$, and $5.02\times10^3 \pm 0.96\times10^3$) cfu/g, receptively and for control was ($9.13\times10^4 \pm 2.01\times10^4$) cfu/g.

Table 10: Analysis of variance (ANOVA) of TABC in the examined chicken meat samples

Source of variance	D.F	S.S	M.S	F.value
Total	63	687983.29		
Between Feeding (F)	3	370204.51	123401.49	29.47 ++
Between Tissues (T)	1	70473.24	70473.24	16.83 ++
(F) × (T) interaction	3	12813.39	4271.13	1.02 NS
Error	56	234492.15	4187.36	

D.F = Degrees of freedom ++ = High significant differences (P<0.01) S.S = Sum squares NS = Non significant differences M.S = Mean squares

Table 11: Influence of supplementation of thymol and carvacrol on coliform count (cfu/g) in the examined samples of chicken meat

Tissues	Thigh			Breast		
Groups	**Min**	**Max**	**Mean ± S.E***	**Min**	**Max**	**Mean ± S.E***
Control	5.1×10^3	6.8×10^4	$1.17\times10^4 \pm 0.20\times10^4$	2.0×10^3	3.7×10^4	$9.03\times10^3 \pm 2.14\times10^3$
A	3.9×10^3	2.7×10^4	$8.46\times10^3 \pm 1.51\times10^3$	1.4×10^3	1.6×10^4	$5.10\times10^3 \pm 0.66\times10^3$
B	1.5×10^3	8.2×10^3	$2.91\times10^3 \pm 0.49\times10^3$	9.0×10^2	4.5×10^3	$1.83\times10^3 \pm 0.29\times10^3$
C	7.0×10^2	5.9×10^3	$1.75\times10^3 \pm 0.27\times10^3$	3.0×10^2	4.1×10^3	$1.26\times10^3 \pm 0.18\times10^3$

S.E* = standard error of mean Total coliform count mean values for group A, B, and C for thigh muscle samples were ($8.46\times10^3 \pm 1.51\times10^3$, $2.91\times10^3 \pm 0.49\times10^3$ and $1.75\times10^3 \pm 0.27\times10^3$) cfu/g ,receptively and for control was ($1.17\times10^4 \pm 0.20\times10^4$) cfu/g .

Table 12: Analysis of variance (ANOVA) of coliform count in the examined chicken meat samples

Source of variance	D.F	S.S	M.S	F.value
Total	63	168210.98		
Between Feeding (F)	3	73951.72	24650.61	18.22 ++
Between Tissues (T)	1	15653.48	15653.48	11.57 ++
(F) × (T) interaction	3	2841.13	947.05	0.70 NS
Error	56	75764.65	1352.94	

D.F = Degrees of freedom ++ = High significant differences (P<0.01) S.S = Sum squares NS = Non significant differences M.S = Mean squares

Table 13: Influence of supplementation of thymol and carvacrol on Staphylococcus counts (cfu/g) in the examined samples of chicken meat

Tissues	Thigh			Breast		
Groups	**Min**	**Max**	**Mean ± S.E***	**Min**	**Max**	**Mean ± S.E***
Control	5.1×10^3	6.8×10^4	$7.17\times10^3 \pm 1.63\times10^3$	2.0×10^3	3.7×10^4	$4.32\times10^3 \pm 0.75\times10^3$
A	3.9×10^3	2.7×10^4	$3.95\times10^3 \pm 0.42\times10^3$	1.4×10^3	1.6×10^4	$1.60\times10^3 \pm 0.28\times10^3$
B	1.5×10^3	8.2×10^3	$1.44\times10^3 \pm 0.25\times10^3$	9.0×10^2	4.5×10^3	$8.87\times10^2 \pm 1.45\times10^2$
C	7.0×10^2	5.9×10^3	$9.18\times10^2 \pm 2.01\times10^2$	3.0×10^2	4.1×10^3	$5.93\times10^2 \pm 0.80\times10^2$

S.E*=standard error of mean Total staphylococcus count mean values for group A, B, and C for thigh muscle samples were ($3.95\times10^3 \pm 0.42\times10^3$, $1.44\times10^3 \pm 0.25\times10^3$ and $9.18\times10^2 \pm 2.01\times10^2$) cfu/g, receptively and for control was($7.17\times10^3 \pm 1.63\times10^3$) cfu/g.

Table 14: Analysis of variance (ANOVA) of Staphylococcus count in the examined chicken meat samples

Source of variance	D.F	S.S	M.S	F.value
Total	63	114045.39		
Between Feeding (F)	3	45027.59	15009.24	14.69++
Between Tissues (T)	1	9011.65	9011.65	8.82 ++
(F) × (T) interaction	3	2789.30	929.77	0.91 NS
Error	56	57216.85	1021.73	

Table ١5: Influence of supplementation of thymol and carvacrol on fungal count (cfu/g) in the examined samples of chicken meat

Tissues	Thigh			Breast		
Groups	**Min**	**Max**	**Mean ± S.E***	**Min**	**Max**	**Mean ± S.E***
Control	7.0×10^2	5.2×10^3	$1.56\times10^3 \pm 0.21\times10^3$	5.0×10^2	4.1×10^3	$1.02\times10^3 \pm 0.15\times10^3$
A	4.0×10^2	3.8×10^3	$9.89\times10^2 \pm 2.09\times10^2$	3.0×10^2	2.2×10^3	$7.64\times10^2 \pm 1.36\times10^2$
B	3.0×10^2	1.9×10^3	$8.01\times10^2 \pm 1.76\times10^2$	1.0×10^2	1.6×10^3	$4.28\times10^2 \pm 0.55\times10^2$
C	1.0×10^2	9.0×10^2	$5.31\times10^2 \pm 0.82\times10^2$	1.0×10^2	6.0×10^2	$2.74\times10^2 \pm 0.49\times10^2$

S.E* = standard error of meat Total fungal count mean values for group A, B, and C for thigh muscle samples were ($9.89\times10^2 \pm 2.09\times10^2$, $8.01\times10^2 \pm 1.76\times10^2$ and $5.31\times10^2 \pm 0.82\times10^2$) cfu/g, receptively and for control was($1.56\times10^3 \pm 0.21\times10^3$).

Table ١6: Analysis of variance (ANOVA) of fungal count in the examined chicken meat samples

Source of variance	D.F	S.S	M.S	F.value
Total	63	75764.97		
Between Feeding (F)	3	23281.63	7760.57	9.36++
Between Tissues (T)	1	4734.28	4734.28	5.71 ++
(F) × (T) interaction	3	1318.34	439.41	0.53 NS
Error	56	46430.72	829.12	

D.F = Degrees of freedom ++ = High significant differences (P<0.01) S.S = Sum squares NS = Non significant differences M.S = Mean squares

3. Results and Discussion

The results revealed that the sensory evaluation of all groups is excellent containing external aspects, odor, color, and muscular elasticity of thigh and breast muscles. It means thymol and carvacrol supplementation caused excellent sensory evaluation.

The results revealed that thymol and carvacrol lowered pH mean parameter of breast and thigh muscles with a high significant difference when compared to the control group shown in Table (5). pH decreased may be due to the antioxidant effect of thymol and carvacrol as reported that pH is one of the factors that is associated with lipid oxidation in meat (Lee et al., 1996). Also, the antioxidant effect of thymol and carvacrol decreased free radicals which may cause pH to decrease.

Thymol and carvacrol supplementation led to decrease TVN values of samples with significant differences when compared with the control group which means it decreased protein oxidation in poultry meat. This proved that thymol and carvacrol have high antioxidant activity as Luna (2010) reported.

Lipid oxidation of meat samples was determined by the analysis of 2-TBA reactive substances according to Nielson et al (1991). Thymol and carvacrol supplementation lowered TBA values in meat with a highly significant difference when compared with the control group which means it decreased lipid oxidation. Those results agree with Luna (2010). This has supported that thymol and carvacrol are antioxidant as Yanishlieva (1999) reported. It was suggested that the high antioxidant activity of thymol and carvacrol could be by the possibility of blocking radical chain process through interaction with peroxide radical (Luna et al., 2010).

Adding thymol and carvacrol to meals of broiler chickens lowered total aerobic bacterial count in thigh and breast muscle samples with high significant differences when compared with the control group. That support the antibacterial effect of thymol and carvacrol which may be because they are strong active respiration and protein inhibitors of bacterial and fungal growth as Vasquez et al (2001) reported.

Thymol and carvacrol lowered Coliforms in thigh and breast muscles of meat samples with a significant difference when compared with the control group. Those results agree with Xu (2008) who reported that thymol and carvacrol have an inhibitory effect against *E coli*.

Thymol and carvacrol supplementation decreased *Staphylococcus* mean value of thigh and breast muscle with a significant difference when compared with the control group as shown in table (15). The antibacterial effect of thymol and carvacrol was supported in this study as Botsoglou (2002) reported. Also, these results agree with Nostro (2007) who reported the antibacterial effect of thymol and carvacrol against *S. aureus* and *S. epidermis* strains.

Thymol and carvacrol supplementation decreased the fungal count of thigh and breast muscle samples with a high significant difference when compared with control. This supports the antifungal effect of thymol and carvacrol and this agrees with Ahmad (2011) who reported that thymol and carvacrol have an antifungal effect by disrupting ergosterol biosynthesis and membrane integrity.

Conflict of interests

The authors have not declared any conflict of interests.

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