

CHAMOMILE FLOWER EXTRACT AS NATURAL DIETARY GROWTH PROMOTER AND ANTIOXIDANT FOR BROILER CHICKENS

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ABSTRACT

The present study was conducted to investigate the effect of dietary supplementation of chamomile flower extract (CFE) on broilers' performance, carcass traits and meat quality under refrigerated storage conditions. Chamomile flower extract was prepared by maceration method using ethanol as solvent. The CFE was prepared in powder form. The CFE was found to have a total phenol content (41.02 mg gallic acid equivalent /g) and total antioxidant capacity (222 mg ascorbic acid equivalent/g). *In vitro* examination has proved that CFE had growth-inhibiting effect on certain Gram positive and Gram negative bacteria. In the growth trial, three corn-soybean meal-based diets (starter, grower and finisher) were formulated and fed to the control group of broiler chickens during the age intervals of 1-14, 14-28 and 28-42 days, respectively. A second group of birds was fed on the same three diets supplemented with 75 ppm CFE. The results showed that final body weight and weight gain of birds fed the CFE-supplemented diet were significantly higher than those of the control group. Also, feed intake, dressing weight and total edible parts were positively affected as a result of feeding the CFE-supplemented diet but feed conversion ratio and giblets' weight were not affected. The estimated value of European production efficiency factor for the tested extract was higher than that of the control group. The percentage of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was higher while the TBARS value was lower in meat of broilers fed the CFE-supplemented diet than those of the control birds. It is concluded that added dietary CFE has a positive effect on broilers' performance and can act as a potent preservative of refrigerated chicken meat; thus, dietary addition of CFE may prolong the shelf life of meat.

Keywords: *Chamomile flower; performance, antibacterial, antioxidant, meat quality, broilers.*

INTRODUCTION

Poultry meat has become primary source of animal protein, especially after the rise in prices of other protein sources in Egypt. Chickens are usually preferred over other protein sources for their taste and texture. Maintaining the quality of chicken meat is limited because the lipid oxidation and microbial growth lead to meat spoilage (Racanucci *et al.*,2008). Oxidation products are considered the main reason of quality damages of meat even under refrigerated conditions and thereby lead to decreasing the shelf life of poultry meat (Tavárez *et al.*,2011). The high content of polyunsaturated fatty acids and low level of natural antioxidants in chicken meat may lead to quality deterioration by lipid oxidation during storage. Microbial growth on meat depends on the slaughter and storage conditions (Dave and Ghali, 2011). On the other hand, the researches in this point are focusing on reducing lipid oxidation and microbial growth by addition of chemical or natural antioxidants and antimicrobial in poultry diets (Avila-Ramos *et al.*,2013; Babuskin *et al.*,2015). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) have been widely used, however these synthetic

chemicals have shown carcinogenic effects (Avila-Ramos *et al.*,2013 ; El Abed *et al.*,2014; Marzoni *et al.*,2014). Using synthetic antioxidants has been refused by many countries because of their toxicological effects and suspected carcinogenic potential (Spigno and De Faveri, 2007). Nowadays researches are directed towards the use of natural antioxidant instead of synthetic chemicals to avoid their negative aspects (Padam *et al.*, 2014). Plant extracts which contain some bioactive compounds have very effective antioxidant activities compared to synthetic antioxidants (Zeković *et al.*,2014). Plant extracts have been studied as natural antioxidants and antimicrobials that can be used as natural feed additive to improve the growth performance and inhibit spoiling microbes and to extend the shelf-life of meat.

Chamomile (*Matricaria chamomilla*) is one of the important medicinal herbs that have been used in herbal remedies for thousands of years, known in ancient Egypt (Singh *et al.*,2011). In Egypt, the total area of chamomile is 4,916 ha which represents 13% of the total area with medicinal and aromatic plants; 77% of chamomile was in Fayoum governorate (Santucci *et al.*, 2013). The productivity of one feddan per harvest is approximately 350 - 500 kilograms of dry herb. There are approximately

50,000 Chamomile plants in each feddan (FAO, 2005). The scientific name of chamomile is *Matricaria chamomilla* and it belongs to the *Asteraceae* family (Singh *et al.*, 2011). Chamomile's main active constituents are chamazulene, apigenin, and bisabolol. The aim of the study was to investigate the effect of dietary inclusion of chamomile flowers extract on broilers' performance, meat quality and oxidative parameters of chicken meat under refrigerated storage and Economic efficiency.

MATERIALS AND METHODS

Preparation of chamomile flowers extract: Chamomile plants were collected and the leaves were separated from the flowers. Chamomile flowers were washed and dried then ground into powder. Then, 25 g of the flowers' powder was extracted using 250 ml of 80% ethanol at room temperature by maceration overnight, with occasional stirring (El-Chaghaby *et al.*, 2014). The extract was then filtered and the solvent was evaporated to obtain the dried chamomile flower extract (CFE).

GC/MS analysis of chamomile flower extract: Gas Chromatography/Mass analysis of chamomile flower extract (CFE) was performed using SHIMADZU GC/MS-QP5050A system and the main constituents of the extract were identified by comparing their retention times and mass fragmentation patterns with the GC/MS spectral database library.

Determination of total antioxidant capacity and total phenolic content of the extract: The total antioxidant activity of chamomile flower extract (CFE) was determined using the phosphomolybdenum method according to the procedure described by Prieto *et al.* (1999). The total antioxidant capacity was expressed as mg ascorbic acid equivalent/ g extract (mg AAE/g extract). The total phenolics of the extract were estimated spectrophotometrically using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). The results were calculated as gallic acid equivalent in mg per g of extract using gallic acid standard curve.

Determination of the antibacterial activity of chamomile flower extract: The antibacterial activity of the chamomile flower extract (CFE) was determined based on the inhibition zones using the disc diffusion method of Bauer *et al.*, (1996). Briefly, 100 μ l of bacterial suspension ($\sim 10^8$ cells/ml) was spread onto agar in Petri plates. Then an aliquot of the extract was pipetted on a sterile paper disc (Whatman No. 1, 5.5 mm paper disc) on the agar surface. Standard discs of "BMD 50" (feed grade antibiotic containing bacitracin methylene disalicylate) served as positive control. The plates were inverted and incubated for 18 h at 37 °C and the inhibition zones were determined by measuring the

diameter of the clear zone of inhibited growth around each disc and recorded (mm).

Chicken growth experiment: A total number of 150 Ross chicks (one day old) were randomly divided into two groups of three replicates each. Each replicate (consisted of 25 birds) was kept in a floor pen. The first group was assigned as a control group without any feed additive. The second group was supplemented with 75g CFE/ton feed as a natural growth promoter and antioxidant (this supplementation dose was chosen based on the results of the antibacterial test that is performed at the beginning of this work). The diets were formulated to meet the nutrient requirements of the broiler chicks as recommended by the Ross broiler management guide (2014). The diets were calculated based on NRC (1994) analytical values of feedstuffs. The starter diets contained 23% CP and 3000 kcal ME/kg, the grower diet contained 21.5% CP and 3100 kcal ME/kg and the finisher diet contained 19.5% CP and 3200 kcal ME/kg. The diet formulation during the starter, grower and finisher periods are given in Table 1. Average live body weight, body weight gain, feed consumption and feed conversion ratio were calculated at 15, 28 and 42 days of age. Mortality was also monitored and recorded daily.

Intestinal bacterial count: To determine the total count of intestinal bacteria, the intestines were collected at day 21 and day 42 according to the method described by Proietti *et al.* (2009). The samples were collected in sterile bags and cooled until their delivery to the laboratory for bacterial count examination. The total aerobic bacterial count was determined using a standard plate count agar medium. The plates were incubated at 30°C, aerobically, for 24 - 48h. All the data are expressed as Log 10 CFU/g.

Measurement of antioxidative properties of chicken meat: At the end of the growth period, (42 days old), three chickens per replicate were randomly selected, slaughtered and manually eviscerated. The carcasses were cut and samples of breast meat were placed in plastic bags and kept refrigerated at 4°C. The antioxidative properties of chicken meat were determined at three refrigerated storage days (day 1, day 4 and day 7). The DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging activity of chicken meat was estimated with the aqueous supernatant prepared from breast and thigh meat samples according to the method reported by Jang *et al.*, (2008). The lipid oxidation of the meat was estimated using the thiobarbituric acid reactant substances (TBARS) test following the procedure described by Racanicci *et al.*, (2008).

Economics of broiler production: At the end of the experimental period (42 days) the European Production Efficiency Factor was calculated, based on the age of broilers at slaughter (days), their average live weight (kg), viability (%) and feed conversion ratio (FCR) (kg feed/kg gain). European Production Efficiency Factors (EPEF) is given by the following equation as proposed by Ross Broiler Management handbook (2014).

$$E = 100 \times \left| \frac{B (K) \times v \text{ it } (\%)}{A (d) \times F} \right|$$

Statistical analysis: Data were statistically analyzed using the CoStat program. Significant differences between the two means of each variable were detected by Student's t test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

GC/Ms analysis results: The GC/Ms analysis results of chamomile flower extract (CFE) are given in Table 2. The analysis of chamomile flower extract showed the presence of several bioactive phytochemical components including pentadecanoic acid which is a palmitic acid methyl ester that was reported to have a potential antioxidant activity (Vijisalar and Arumugam, 2014); α -bisabolol (a monocyclic sesquiterpene alcohol) which was found to have an anti-inflammatory, antibacterial and antifungal properties (Kazemi, 2014) and 4,5,9,10-dehydro- isolongifolene, a sesquiterpene with potent antioxidant properties (Rangasamy and Namasivayam, 2014). Other compounds that were identified in the GC/MS analysis were 4,7-dihydroxy-coumarin and 7-methoxy-coumarin, phenolic compounds that may act as potent metal chelators and free radical scavengers. Coumarins have a variety of important biological activities such as anti-inflammatory, antioxidant, antiviral, antimicrobial and anti-cancer (Al-Majedy *et al.*, 2016). Farnesol was also identified in the GC/Ms analysis of chamomile flower extract. Farnesol was reported by Kazemi, (2014) to have an antibacterial activity. The tested extract was also found to contain the terpene compounds farnesyl- β -D-mannofuranoside, phytol and lavandulol having antimicrobial (Gunasekaran *et al.*, 2012), anticancer (Nayak *et al.*, 2014) and antioxidant activities (Ghaneian *et al.* 2015). Another compound present in the tested extract was 7,8-Dihydro- α -ionone which has an antibacterial activity. It is thus clear that chamomile flower extract is a source of many phytochemicals with diverse biological activities that allow it to be used as a natural antioxidant and antimicrobial.

Total antioxidant capacity and total phenolic content of CFE: Chamomile flower extract were analyzed to determine their total antioxidant capacity and total phenolic content. The total antioxidant capacity of chamomile flower extract was found to be 222 \pm 3.05 mg

AAE/g and its total phenolic content was 41.02 \pm 0.54 mg GAE/g as shown in Table 3. The obtained results are in consistence with the GC/MS results that showed the presence of several compounds with antioxidant activities in the extract. These results indicate that chamomile flower extract can act as a natural antioxidant. This is very important as antioxidants prevent initiation of chain oxidation reactions and continued hydrogen abstraction; they possess mechanisms for binding of transition metal ion catalysts, decomposition of peroxides and radical scavenging antioxidant activity (Subhadradevi *et al.*, 2010).

Antibacterial activity of CFE: The antibacterial activity of CFE against three Gram positive and three Gram negative bacteria was determined by the diameter of inhibition zones and is shown in Table 4. The results revealed that CFE has a strong inhibitory effect on the growth of the six species of studied bacteria. It has also to be noted that the average bacterial inhibitory effect of CFE was 70% compared to the standard antibiotic BMD50 which means that 1 g of BMD50 is equivalent to about 1.43g of CFE. Our results are in agreement with the findings of several authors, who have estimated the antibacterial activity of different types of chamomile extracts (Kordali *et al.*, 2005; Ahameethunisa and Hopper, 2010; Naili *et al.*, 2010). Also the antibacterial activity of CFE was confirmed by the GC/Ms analysis results which proved the presence of several bioactive compounds exhibiting antibacterial activity in CFE.

Growth performance: The results of growth performance in Table 5 showed that the addition of chamomile flower extract (CFE) did not significantly affect the growth, feed intake or feed conversion compared with those of the birds fed control diet during the starter period. Also, there were no significant differences between the feed intake and feed conversion of treatment group and control group during the grower period; while treatment group recorded significantly higher body weight and weight gain compared to control group. During the finisher period, the effect of dietary inclusion of CFE was more pronounced with significant differences ($p < 0.01$). The treatment group had significantly ($p < 0.01$) higher body weight, weight gain and feed intake compared to the control group. While, feed conversion ratio was significantly ($p < 0.01$) better for treatment group compared to the control group. Concerning the total experimental period, it was also noted that treatment group showed significantly higher ($p < 0.01$) body weight, weight gain and feed intake compared to the control group. Concerning feed conversion ratio, the treatment group displayed slightly better values but with no significant difference compared to the control group numerically increased. The inclusion of medicinal plants or herbal extracts in broilers' diet were previously reported to have positive effect on broilers' growth by enhancing feed intake, secretion of

gastrointestinal fluids and improvement of nutrients' digestion and absorption (Marzoni *et al.*, 2014). Our results are also in agreement with those of Wang *et al.* (2008) who reported that dietary inclusion of *Forsythia* suspense extract can improve broilers' performance due to its antioxidant positive effect on health and nutrient digestibility.

Carcass characteristics: Means of carcass traits (g) of 42-day-old broiler chicks as affected with the dietary treatments are illustrated in Table 6. The treatment group had heavier live body weight ($P < 0.05$) compared to the control group. Concerning total edible parts (TEP) results revealed that the absolute weight of TEP was significantly higher in the treatment group than that of the control group. Concerning the dressing weight, chicks fed the CFE-supplemented diet achieved significantly higher dressed weight as compared to the control group. Regarding the absolute weight of giblets (liver, gizzard and heart), results revealed that the differences among the CFE- supplemented and control groups in these traits were insignificant.

Intestinal bacterial count: The total aerobic bacterial count in the small intestine of the chickens in the control and CFE treated groups are shown in Table 7. It was noticed that supplementing the diet with CFE caused a highly significant ($P < 0.01$) reduction in the total intestinal bacterial count of the chickens as compared to the control group. This result is in consistence with many previous studies confirming that the pathogenic intestinal microflora is controlled by the direct antimicrobial action of phytochemical extracts (Jamroz *et al.*, 2003).

Antioxidative properties of chicken meat: The presence of high amount of polyunsaturated fatty acids in chicken meat makes it highly susceptible to oxidative deterioration (Zhang *et al.*, 2015). According to the food

storage chart provided by the *Food and Drug Administration (FDA)*, chicken meat can be preserved refrigerated (at 4°C) for a period of only two days (FDA, 2015). In the present work, the effect of dietary addition of CFE on the oxidative stability of chicken meat (up to 7 days) was evaluated by determining the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and the thiobarbituric acid reactant substances (TBARS) of breast meat under refrigerated storage conditions.

Table 8 shows the changes in DPPH scavenging activity of chicken meat of the control and CFE supplemented groups at different refrigerated storage days. The DPPH scavenging activities of breast meat from the birds fed diet supplemented with CFE were significantly higher ($p < 0.01$) than those fed the control diet at all investigated storage days (1, 4 and 7). It was observed that by increasing the storage time from day one up to day seven the DPPH scavenging activity of chicken meat fed the control diet decreased, whereas the chicken meat of the CFE-supplemented group showed no significant ($p > 0.05$) reduction in their DPPH scavenging activities up till day seven.

TBARS values of breast meat samples of the control and CFE treated group are also given in Table 8. The results indicated that during the first day of storage (day one) the TBARS value of breast meat of CFE group was significantly lower ($p < 0.01$) than that of control group. Upon increasing the storage period (at d4 and d7), the supplementation of broiler diet with CFE resulted in significantly lower ($p < 0.01$) TBARS values for breast meat compared to those fed the control diet. The results showed that the dietary addition of CFE into broilers' diet had a positive effect on delaying the lipid oxidation of broilers' meat up to seven days of refrigerated storage.

Table 1. Starter, grower and finisher Ross diets composition and analysis.

Ingredients (%)	Starter (1-14 days)	Grower (15-28 days)	Finisher (29-42 days)
Yellow Corn 7.9% CP	57.720	57.950	60.00
Soybean meal 46% CP	28.500	28.500	31.00
Corn Gluten meal 60.5% CP	7.485	5.359	-
Corn oil	1.54	3.60	5.135
Di-Calcium phosphate	2.030	2.030	1.600
CaCO ₃ (38% Ca)	1.140	1.140	1.000
Premix *	0.400	0.400	0.400
NaCl	0.300	0.300	0.300
Choline Chloride	0.075	0.075	0.075
DL-Methionine	0.490	0.300	0.310
L-Lysine - HCl	0.320	0.346	0.180
Total	100.00	100.00	100.00
	Calculated (as fed: NRC,1994)		
Crude protein %	23.00	21.50	19.50

Metabolizable energy Kcal/kg	3000	3100	3200
Calcium %	0.97	0.97	0.83
Available phosphorus %	0.50	0.49	0.40

*Premix supplied per Kg of diet: Vit. A, 12000 I.U., Vit.D₃, 2000I.U. ; Vit.E, 10mg ;Vit.K₃, 2mg; Vit.B₁, 1 mg; Vit.B₂, 5 mg; Vit.B₆, 1.5 mg; Vit.B₁₂, 10 µg; Biotin, 50ug; Choline chloride,500mg; Pantothenic acid , 10mg;Niacin,30mg;Folic acid,1mg; Manganese, 60mg; Zinc,50mg; Iron,30mg;Copper,10mg;Iodine,1mg; Selenium,0.1mg and Cobalt,0.1mg (According to Ross broiler management guide (2014).

Table 2. GC/MS analysis of chamomile flower extract.

Retention time (min.)	Chemical compound	Compound nature	Area %
17.4	7-methoxy- coumarin	Benzopyrone	1.460035
21.43	Phytol	acyclic diterpene alcohol	1.61324
24.23	Farnesol	sesquiterpene alcohol	1.981719
21.79	(±)-Lavandulol	monoterpene alcohol	1.998702
16.4	4,7-dihydroxy- Coumarin	Benzopyrone	2.886993
19	4,5,9,10-dehydro -Isolongifolene	Sesquiterpene	4.36823
21.64	α-Bisabolol	sesquiterpene alcohol	8.836129
21.6	7,8-Dihydro-α-ionone	unsaturated ether	15.54966
20.07	Pentadecanoic acid	palmitic acid methyl ester	21.78434
24.411	farnesyl-β-D-mannofuranoside	Terpene	28.14237

Table 3. Total antioxidant capacity and total phenolic content of CFE.

Total antioxidant capacity (mg AAE/g)	222±3.05
Total phenolic content (mg GAE/g)	41.02±0.54

Mean± standard deviation

Table 4. *in vitro* antibacterial activity of CFE.

Bacterial species (Gram reaction)	CFE	BMD 50	Inhibition
	Inhibition zone diameter (mm)		percentage* (%)
<i>Bacillus subtilis</i> (G+)	13	17	76.47
<i>Staphylococcus aureus</i> (G+)	12	14	85.71
<i>Streptococcus faecalis</i> (G+)	13	20	65.00
<i>Escherichia coli</i> (G-)	13	21	61.90
<i>Neisseria gonorrhoeae</i> (G-)	13	20	65.00
<i>Pseudomonas aeruginosa</i> (G-)	13	19	68.42

*The inhibition percentages are calculated by comparing the inhibition zone caused by CFE to that of BMD50

Table 5. Effect of dietary inclusion of (75 ppm) chamomile flower extract (CFE) on broilers' performance from day-old to 6 weeks of age.

Traits	Treatment	Control Basal diet	Treatment CFE-diet	p-value	Sig.
		(1-14 day)			
Body weight (g)		331.00±1.53	333.67±3.84	0.5543	NS
Body weight gain (g)		290.00±2.65	294.67±4.73	0.2099	NS
Feed intake (g)		382.00±5.13	386.67±11.55	0.6087	NS
Feed conversion ratio		1.31±0.0088	1.32±0.0088	0.8025	NS
		(15-28 day)			
Body weight (g)		1160.33±11.35	1195.00±2.31	0.0402	*

Body weight gain (g)	829.33±17.10	861.00±8.19	0.0444	*
Feed intake (g)	1376.00±19.52	1418.67±3.18	0.0972	NS
Feed conversion ratio	1.66±0.0033	1.64±0.0115	0.2378	NS
(29-42 day)				
Body weight (g)	2240.00±5.13	2403.76±2.6	0.0001	**
Body weight gain (g)	1079.67±11.37	1209.67±2.08	0.0001	**
Feed intake (g)	2096.67±14.17	2294.33±4.91	0.0002	**
Feed conversion ratio	1.94±0.0067	1.89±0.0033	0.0026	**
(1-42 day)				
Body weight (g)	2240±5.13	2403.67±2.6	0.0001	**
Body weight gain (g)	2199.00±8.89	2365.33±8.74	0.0001	**
Feed intake (g)	3854.67±21.76	4099.67±9.53	0.0005	**
Feed conversion ratio	1.75±0.0058	1.73±0.0033	0.0668	NS

± Standard deviation (SD)

Table 6. Effect of dietary inclusion of chamomile flower extract (CFE) on absolute carcass characteristics of broilers at 6 weeks of age.

Traits	Control	Treatment	p-value	Sig
Body weight (g)	2248.33±7.26	2356.66±7.26	0.0005	**
Total edible weight (g)	1969.00±6.68	2082.00±6.68	0.0003	**
Dressed weight (g)	1846.00±6.54	1952.66±6.54	0.0003	**
Liver weight (g)	49.33±1.76	51.66±1.76	0.4025	NS
Gizzard weight (g)	64.00±1.68	68.00±1.68	0.1682	NS
Heart weight (g)	9.66±0.08	9.66±0.08	1.000	NS

± Standard deviation (SD)

Table 7. Total bacterial count (Log 10 CFU/g) in intestine of 21 and 42-day-old chickens fed CFE supplemented diet.

Age	Control	Treatment	p-value	Sig
21Day	7.38±0.0058	6.8767±0.0088	0.0001	**
42 Day	7.4933±0.0145	7.067±0.0088	0.0001	**

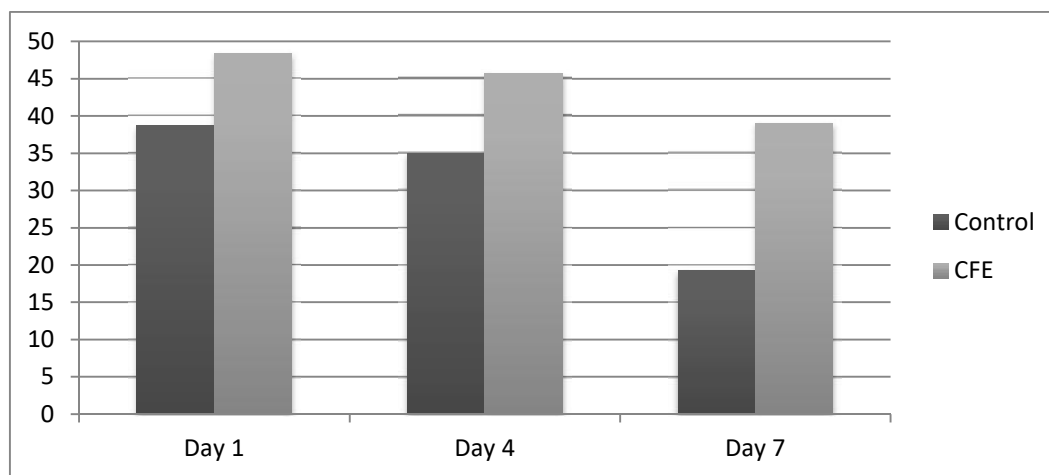
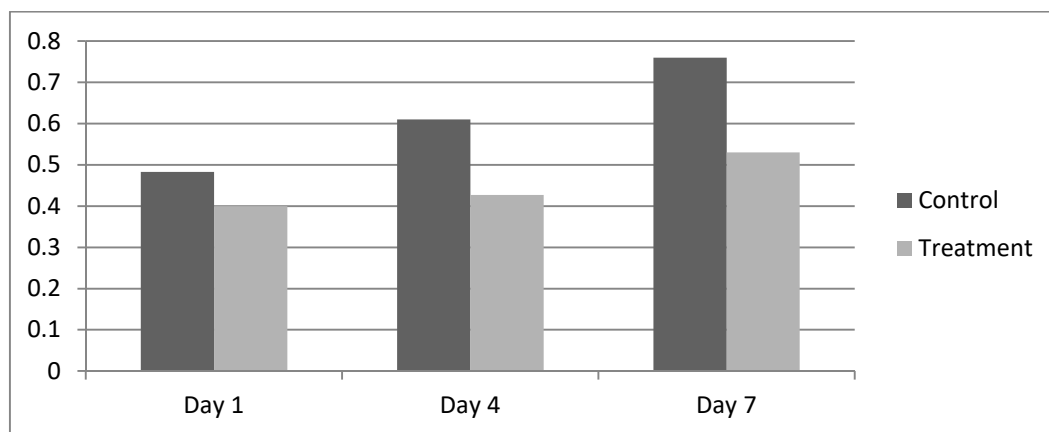
Table 8. DPPH scavenging activity (%) and changes in TBARS of chickens' meat fed diet supplemented with CFE under refrigerated storage.

DPPH scavenging activity (%)	Day 1	Day 4	Day 7
Control	38.67±0.33	35.00±1.15	19.33±0.88
Treatment	48.33±0.88	45.67±0.88	39.00±1.15
p-value	0.0005	0.0018	0.0004
Significance	**	**	**
TBARS (mg of malondialdehyde/kg of meat)			
Control	0.483±0.006	0.610±0.010	0.760±0.010
Treatment	0.400±0.010	0.427±0.006	0.530±0.053
p-value	0.0002	0.0001	0.0018
Significance	**	**	**

Table 9. Effect of dietary inclusion of Chamomile flower extract (CFE) on (EPEF^{*}) of broilers at 6 weeks of age.

Traits	Control	Treatment
Live weight (g)	2240	2403.67
Feed conversion ratio	1.75	1.73
Livability (%)	97.33	96.00
EPEF [*]	296.62	317.48

^{*}European production efficiency factor.

**Figure 1.** Effect of supplemented CFE under refrigerated storage time on DPPH scavenging activity of chicken breast meat [control, CFE (75ppm CFE)].**Figure 2.** Effect of supplemented CFE under refrigerated storage time on change in TBARS of chicken breast meat [control, CFE (75ppm CFE)].

Economics of broiler production: The evaluation of results in Table 9 indicated that the estimated value of EPEF was higher in the CFE group than that of the control group.

Conclusion: It can be concluded that the dietary inclusion of chamomile flower extract as a natural antioxidant has positive effect on broilers performance, meat quality and also improves the chicken meat oxidative stability during refrigerated storage up to 7 days. Further studies should explore the physiological

aspects to specify the optimum levels of dietary inclusion of chamomile flower extract.

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