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Effects of Active Composition of Garlic Powder, Clover Powder and Savory Essence of Khuzestan (*Satureja khuzistanica*) on Fat Reduction, Carcass Quality and Improvement of Oxidative Stability and Shelf-life of Broilers Meat

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Abstract

This experiment was conducted to determine the effects of active composition of different levels of garlic powder, clover powder and Satureja of Khuzestan (SK) essential oils on performance traits, fat reduction, carcass quality, meat quality and quantity, shelf-life, carcass traits, functional components, the concentration of the blood and immune system, metabolites and carcass antioxidant properties. A total number of 560 one-day old chicks of commercial Ross strain-308 were allocated in a completely randomized design with 7 treatments and 4 replicates of 20 birds per each replicate. Treatments were: 1. Control treatment (without additive). 2. Treatment containing garlic powder in two levels (2% and 4% of diet), 3. Clover powder treatment on two levels (5% and 10% food ration), and 4. *Khuzestan satureja* (Savory) essential oil in two levels (400 and 500 mg/kg). At 38 days of age, one bird from each replicate was randomly slaughtered and 30 cc blood and serum samples were taken from the jugular vein with syringes to evaluate the immune system, blood metabolites and anti-oxidant condition of broilers. The results showed that the impact of active constituents of garlic powder, clover powder and essential oils of savory for different treatments on the amount of dry matter, crude protein, crude fat and water holding capacity (WHC) and fat peroxidation of breast tissue at different times were significant (P<0.05). The results showed that the level of 10 percent clover powder relative to other treatments leads to reduction of WHC and the percentage of fat breast tissue. With regard to the different times of keeping meat Chest at freezer (0, 24 and 48 hours) the amount of breast tissue on fat peroxidation showed statistical differences. Production of Malonedialdehyde (MDA) in the breast meat did not show significant difference in experimental treatments (P<0.05). Preservation at different times showed significant impact on the amount of MDA production of breast meat (P<0.05). After a 48-hour freezing, the concentration of Malone aldehyde in breast meat of birds which received different levels of clover powder were less than the birds fed with essential savory and garlic powder (P<0.05).

Keywords: Clover powder; Garlic powder; *Khuzestan satureja* essence; Malonedialdehyde; Broilers

Introduction

Essential oils of herbaceous with respect to the levels of consumption, production period, age of the bird and slaughtering time have different effects on meat quality [1]. For example, addition of garlic powder [2] caused reduction of crud fat in chicken meat [3]. The existence of unsaturated fatty acids in fat content of poultry carcass increased the susceptibility of their meat in relative to oxidation during preservation time and changes the flavor of these products [4]. Supplementation of plant extract to poultry diet can have positive effect on carcass quality as well as the properties of stored meat quality [5]. Addition of flavonoids And the trepans (Azulan) contained in the essential oil have antioxidant properties which can influence on carcass quality after slaughtering and even as freeze during preservation period [6,7].

Meat peroxidation measurement can be performed on the base of primary oxidation such as calculation of the lipid hydro peroxidation (LHP), secondary oxidation by measuring malonedialdehyde (MDA) or products derived from cholesterol oxidation (COP). Production of MDA to some extent explains the oxidative spoilage. Among the different methods for calculating the MDA, the method of thiobarbituric acid (TBA) is applied for the calculation of lipids oxidation rate in animal tissues. This experiment is established on the base of amount of light absorption complex pink color obtained from the reaction one molecule MDA with two molecules TBA. This method is rapid, simple, and the most common form is used for the calculation of the MDA in a sample of meat.

The Role of Different Plant Essences in the Metabolism of Fats

It is reported that utilization of various solvents of savory plant can be considered as a natural, cheap, accessible and potential antioxidant source for food and drug purposes [8]. Shirali reported that phenolic and flavonoid composition, carvacrol and Thymol that constitute the major part of savory essential oils cause antioxidant properties in this plant. This property prevents of lipids being oxidized, heart coronary disease and cancer [9].

The ability of water preservation is one of the most important characteristics of meat. The major part of water in muscle is located inside the myofibrils, between myofibrils and the muscle cell membrane (Sarcolma) and between muscular cells and between muscular masses [10].

Some herbal plants like savory, because of the polyphenol compounds especially carvacrol decrease fat in the abdominal cavity and blood lipoprotein [11]. Addition of the essential oil of *khuzestanian satureja* to drinking water of broilers under heat stress, with increase enzyme antioxidant activity and reduced peroxidation of lipid, causes increasing anti-oxidative potential of muscle [12]. Utilization of natural antioxidants in broilers diet causes reduction of lipids peroxidation in food and different tissues of their body [13]. The use of phytogenic (mixture of some plants) increases anti-oxidative potential of chicken breast muscle [14,15].

Savory herbal plants, because of having phenolic compounds (carvacrol) reduce abdominal fat cavity and lipids of blood protein in chicken [16]. Kamkar et al. reported that essence and aquatic and alcoholic extracts of savory can be considered as a source of natural antioxidants, accessible, cheap and potential for food and medicinal purposes. Recently much attention has been received towards the researches about reduction of the amount of fat and cholesterol and also changing the composition of fatty acids in poultry meat. The most important options to modify the composition of chicken meat are nutritional techniques like adding herb essences to the diets. Changing the taste and smell of meat is due to the reduction of fat and increasing the sustainability of their sensitivity to oxidation for changing the composition of fatty acids of poultry carcasses. Therefore, numerous researches have been conducted to increase the sustainability of the chicken fat through different sources, like addition of essences of herbal plants to poultry feed. The desire of consumers for chicken meat free of antibiotics residues as well as the continuation use of substances derived from medicinal plants or materials to support phytogenic and phytobiotics and the wide field of research related to this issue. Phytogenic materials in addition to the effect on reduction of fat and especially cholesterol of chicken meat, effects on microbial and antioxidant properties of meat [17]. Khosravinia et al. in their studies indicated that the herbal plants of savory due to polyphenol compounds contents (carvacrol) resulting in reduction of fat abdominal cavity and lipids of blood proteins [15].

To determine the effects of active compounds of garlic powder, clover powder and essential oils of *Satureja khuzestanica* on fat reduction, quality of carcass and improvement of quantitative and qualitative and shelf-life traits of meat in broilers, an experiment was conducted on total number of 560 one-day-old commercial strains of Ross 308 in a completely randomized design with 7 treatments and 4 replications and 20 birds in each pen. The treatments were 7 types of diets, including: 1. Control treatment (without additives). 2. Garlic powder with two levels (2 and 4% of diet), 3. Clover powder with two levels (5 and 10% of diet), 4. Savory essential oil with two levels (400 and 500 mg/kg). Diets were balanced for production periods of 0 to 10, 11 to 24 and 25 to 42 days, based on feed requirements catalogue (2014) of Ross strain 308. The feed ingredients of experimental diets in different periods of starter, grower and finisher are demonstrated in Table 1 respectively. The metabolizable energy, crude protein and other nutrient requirements were formulated according to Ross strain 308 recommends and nutrient composition of experimental feed diets were balanced by the use of analysis tables of Iran feedstuffs (Animal Science Research Institute of Iran).

Method of essence extraction

At first upper areal part of dried savory plant grinded by miller and then for extraction of essence the method of distillation with water and clinger utensil was used. One hundred grams of dried plant powder was weighted and poured in a round bottom balloon with the capacity of 1 liter (about two third of balloon), and after that some water was added to it and the balloon was joined to clinger until the procedure of distillation completed for about 4 hours. After extraction of essence, the process of water removing was performed by the use of dried sodium sulfate and then the produced material was kept in small dark color glass and preserved in refrigerator.

The efficiency of essence was 1.7% (1.7 ml essence out of 100 g dried plant).

Determination of chemical composition

The derived essence was injected to GCMS instrument in order to determine the type of its constituents. The gas chromatography utensil was Hewlet Packard 689 N type with a pillar of 30 meters length, internal dimension of 0.25 mm and thickness of 0.25 um (HP-5MS type). The programmed temperature of the shaft was adjusted as: initial temperature of oven was 50°c, final temperature 150°c and gradient temperature of 2.5°c/min, increasing temperature up to 260°c with speed of 10°c/min. and remaining at this temperature for 5 min. and finally increasing temperature up to 320°c with speed

of 15°c/min. and stopped at this temperature for 3 minutes. The temperature of injection box was 250°c and helium gas was used as carrier gas with flow speed of 1.2 ml/minute **(Table 1).**

Table 1: Feed ingredients constituent of control diets and savory essence under 3 periods of production in broilers*.

Ingredients (%)	Starter (1-10 days)	Grower (11- 24 days)	Finisher (25-42 days)
Corn	54.32	0.58	0.66
Soybean meal	39.8	22.35	0.29
Oil soya	2.15	30.3	0.2
Fish meal	0.8	70	65
Dicalcium phosphate	1.75	65.1	30.1
Vitamin permix **	0.25	25	25
Mineral permix ***	0.25	25	25
Salt	0.23	23	20
DL-Methionine	0.28	20	20
Lysine	0.17	20	15
Calculated composition			
ME (kcal/kg)	2.98	3.1	3.15
Crude protein (%)	23	21.24	19.08
Methionine (%)	0.51	0.5	0.47
Methionine +cysteine(%)	0.95	0.81	0.76
Lysine (%)	1.29	1.23	1.04
Threonine (%)	0.8	0.71	0.63
Tryptophan (%)	0.35	0.26	0.22
Calcium (%)	0.98	0.9	0.76
Available phosphorus (%)	0.46	0.44	0.37
Sodium (%)	0.2	0.2	0.2
Diet nutrient analysis (%			
Crude protein	-	-	20.35
Crude fiber	-	-	2.66
Crude ash	-	-	5.87
Crude fat	-	-	4.98
Gross energy	-	-	4042.74
Ca	-	-	1.61
P	-	-	0.52

*Savory essential oil at two levels (400 and 500 mg/kg); **Vitamins premix/kg of diet: global, 44000 unit vitamin A (retinol), 7200 universal unit D3, 440 mg E, 40 mg K, 70 mg cubamalin,65 mg thiamin, 320 mg riboflavin, 290 mg pantothenic acid, 1220 mg niacin, 65 mg pyridoxine, 22 mg biotin, and 270 mg choline chloride ***Minerals premix (mg/kg): 99.200 mg of manganese oxide (MnO), 85 mg of zinc oxide (ZnO), 50 mg of iron sulfate (FeSo4) 10 mg copper sulfate (SuSo4 2.0 mg), selenium (NaSO4.H2O) iodine, 13 mg (calcium iodine) and 250 mg of choline chloride.

Determination of qualitative and quantitative characteristics of meat

To evaluate the qualitative and quantitative traits of broilers, at the end of trial two birds weighing close to the mean weight were selected from each pen. After slaughtering and bleeding by jugular vein, birds were de-feathered and eviscerated. The thigh with shank and breast of left part of the body were cut, separated, homogenized and then preserved and kept in freezer at -20°C temperatures.

Qualitative and physical traits such as, dry matter (DM), crude protein (CP), muscle pH, water holding capacity (WHC), tissue texture, meat tenderness and also quantitative traits such as, chemical characteristic (like fat and protein), WHC, livability and stability and peroxidation tests.

The initial and final pH of each sample of breast meat were measured 15 and 24 hr. after slaughtering respectively by pH meter having probe penetrability into the meat tissue directly. After measuring the initial pH, 10 g of breast muscle meat sample of each bird was provided and chilled at -80°C.

To measure and determine the amount of oxidation and peroxidation of fat in samples of breast meat, the method of fast, sensitive and specific thiobarbituric acid was used. To produce oxidation in breast meat samples, the solution of 1.138 milimole of ferric sulfate and 0.368 milimole of ascorbic acid were used [18,19] in such a way that 1 g of breast meat sample was soften and stabilized gently and 1.5 g ferric sulfate and ascorbic acid was added and then kept in incubator with temperature of 93°C at different times of 50, 400 and 450 minutes.

A sample also was considered without oxidation by ferric sulfate soluble and ascorbic acid (zero time) as control sample. After passing the incubation time, the MDA test for samples was performed and 4 ml of TCA solution and 2.5 ml BHD were added to samples. Then the mixtures after 30 seconds with high speed were rotated and at the same time, they were kept in ultrasonic benmary.

After 30 minutes the samples were centrifuged at 3000RPM and then the upper layer of hexane removed and discarded and the aquatic phase was filtered with no.1 watman paper. The aquatic phase reached to volume of 50 ml by TCA solution. Then 3 ml of TBA solution was added to the sample and kept in benmary at temperature of 70°C for 30 minutes.

The samples immediately were dried in iced water. The concentration of produced MDA solution was read by spectrophotometer at wavelength of 521.5 nm. The WHC was measured by centrifuging 1 g of fresh meat sample for 4 min. at 3000RPM. The samples were weighted and then dried in oven with temperature of 70°C for 12 hours and again they were weighted **(Table 2).**

Table 2: The ingredients and composition of garlic and clover powder under 3 periods of production in broilers (%).

Ingredients	Starter (25-42 days)				Grower (11- 24 days)				Finisher (1-10 days)			
	Clover powder		Garlic powder		Clover powder		Garlic powder		Clover powder		Garlic powder	
Corn	54	52.57	0.58	68.59	0.5	0.53	0.57	0.58	0.5	0.53	0.53	0.54
Garlic powder	2	4	0.4	0.2	0	0	4	2	0	0	0.4	0.2
Soybean meal	38.27	0.3	0.31	0.31	72.33	12.35	0.32	68.33	35	37	27.38	27.38
soya oil	2	0.4	0.4	0.4	8.3	40.3	0.3	0.3	5.1	5.1	0.2	0.2
Clover powder	0	0	5	10	0.1	0.5	0	0	10	5	0	0
Fish meal	50	70	61	61	50	70	61	61	70	70	80	80
Dicalcium phosphate	60.1	65.1	40.1	65.1	60.1	65.1	65.1	65.1	62.1	62.1	75.1	75.1
Mineral premix	25	25	25	25	25	25	25	25	25	25	25	25
Vitamin premix	25	25	25	25	25	25	25	25	25	25	25	25
Salt	23	23	23	23	23	23	23	23	23	23	23	23
DL-methionine.	20	20	15	18	20	20	18	18	28	28	28	28
Lysine	20	20	11	15	17	20	15	15	17	17	17	17
Calculated composition (%)												
Metabolizable energy (kcal/kg)	19.3	16.3	5.3	11.3	6.3	8.3	98.2	3.3	2.95	96.2	87.2	92.2
Crude protein	47.21	8.21	52.19	57.19	32.24	0.23	0.2	64.2	93.21	36.22	0.22	41.22
Methionine.	44	47	43	46	48	49	46	47	56	58	58	59
Methionine	69	74	71	75	76	79	75	77	86	89	90	91
Cysteine	-	-	-	-	-	-	-	-	-	-	-	-
Lysine	1.1	9.1	6.1	9.1	15.1	22.1	12.1	16.1	18.1	24.1	26.1	29.1
Threonine	56	62	65	65	67	70	66	69	89	73	74	75
Tryptophan	20	23	23	23	25	26	24	25	25	27	27	28

Calcium	94	97	78	66	96	98	87	87	5.1	98	98	98
Available phosphorus	43	44	39	43	44	44	44	44	45	44	46	46
Sodium	20	20	18	19	20	20	20	20	20	20/0 9	20	20
Analysis composition (%)												
Crude protein	5.21			69.19			16.22			77.2		
Crude fibre	33.3			22.2			32.3			97.3		
Crude ash	88.4			56.5			53.5			18.5		
Raw fat	88.6			56.6			63.7			60.8		
The raw energy of hot working	64.4773			86.462			4.4644			50.3979		
Calcium	64			58			64			55		
P	64			58			64			55		

*Minerals (mg/kg): 99200 mg manganese oxide (MnO), 85 mg of zinc oxide (ZnO), 50 mg of iron sulfate (FeSo4) 10 mg of copper sulfate (SuSO4), 2.0 mg of selenium (sodium slanit), 13 mg of iodine (calcium iodate) and 250 mg of chloride choline. Supplements used in the composition of rations in the kg, has been the following materials: Supplement Vitamins include: global, 44000 unit vit. A, 7200 universal unit d-3, 440 mg E, 40 mg K, 70 mg cubalamin, 65 mg thiamine, 320 mg riboflavin, 290 mg Pantothenic acid, 1220 mg niacin, 65 mg pyridoxine,22 mg biotin and 270 mg choline chloride.

Shelf-life of meat products

In order to perform testing of full fat peroxidation, at the end of experiment, two birds from each pen were killed and the skin was removed from the thigh and then the thigh meat samples placed in plastic bags to measure the extent of MDA as an indicator of oxidative stability in four different times (0, 50, 100, 150 hours) and was preserved at temperature of -20°C in freezer.

Measurement of water holding capacity (WHC)

For the measurement of WHC, one gram of hot fresh meat sample from the breast and thigh were Centrifugal 1500 xg for four minutes. Then the samples weighted and kept in oven at the temperature of 70°C for a period of 24 hours. After taking out the quota from the avon again they were weighted. The water holding capacity was calculated with the help of following equation (Castellini et al.).

WHC (%) = [weight after centrifugation (g) – weight after drying (g)]/initial weight (g) × 100

Measurement of meat malone aldehyde

The rapid, sensitive and specific method of TBA was used to determine fat peroxidation in thigh meat samples. In this method for measuring MDA as markers for fat peroxidation in the sample, the TBA was used by connecting to the MDA produce color and can be measured by the spectrophotometry device [19].

Preparation of reagents: For preparation of reagent different aqueous solutions of TCA (5%), BHT in hexane (0.8%) and TBA (0.8%) were used.

Preparation of standard solution of MDA: The amount of 73.2 mg of tetraothexy propane (TEP) along with 0.1 mg normal Hydrochloric acid has been reached to a volume of 10 mg was kept at the temperature of boiling water for five minutes and then quickly cooled with tap water. For preparation of stored solution (239 mcg MDA in ml), 1.0 ml stored solution of TEP hydrolysis with distilled water has been reached to a volume of 100 ml. For preparation of working solution (2.39 mcg MDA in ml), one ml of distilled water reached to a volume of storage solution 100 ml by distilled water. The proportion of different work and TCA were mixed together. The amount of 3 ml TBA was added to standard pipes and was placed in bin Marie at temperature of 70°C for 30 minutes, and then the samples quickly cooled in ice water. The standard solution was read by spectrophotometer device model Perkin Elmer Lambda in 521.5 nm wavelength. To create oxidation in thigh meat samples, the solution of 1.138 m mole ferric sulfate and 0.368 ml mole of acid Ascorbic were used, so that the value of a typical hot thigh meat was soft and uniform well. Then 1.5 ml of soluble ferric sulfate and Ascorbic acid was added to it and kept in the incubator with temperature of 37°C at different times of 50, 100 and 150 minutes. One sample also without any exposure to oxidation with ferric sulfate and Ascorbic acid (time zero) were considered as control group [19].

After incubation the samples were kept under the MDA test, so 4 ml of TCA solution and 2.5 ml of BHT solution was poured on the samples for 30 seconds with high speed vertexes and put under Ben Mari ultrasound again for 30 seconds, and centrifuged 3000 xg for 3 minutes. Then the upper hexane layer was discarded and the aqueous phase filtered with Watman paper (No1). The aqueous phase was reached to volume of 5 ml with TCA solution. The amount of 3 ml TBA solution was added to the samples and for 30 minutes kept in Ben Marie with the temperature of 70°C. Then the samples quickly cooled in ice water. The MDA concentration due to light absorption at a wavelength of 5.521 nm and compared to a standard curve was calculated.

The mathematical model of the plan is as follows:

$Yij = \mu + \beta i + \varepsilon ij$

μ = Average of treatments

β i = Effect of experimental treatments

εij = The effect of the experimental error

Data were processed with Excel software and analyzed by the GLM procedure and statistical software of SAS (SAS Institute, 2002). Comparison of the means was performed by the Duncan test at the level of 5 percent.

Results and Discussion

The constituted composition of *Satureja khuzestanica* essence is shown in **table 3**. The major components of SK essence were carvacrol (39.74%) and para-simen (24.5%). Though Sefidcan et al. has reported that the main components of SK are para-simen (39.6%) and carvacrol (29.6%), but the concentration of the components was different from the results of present study. Also Kamalizadeh reported that carvacrol is the most components of the SK essence. Sefidcan et al, reported that the composition available in 3 species of savory indicated that *Satureja mutica* mainly have carvacrol (30.9%) and thymol (26.5%) and essence of *S. maercantha* has para-simen (25.8%) and Limonen (16.3%), and *S. intermediae* has thymol (32,3%) and gama- terpinen (29.3%). Also Kamcar et al. (2013) reported that the results of experiment obtained from 32 compositions of SK overall 98.92% essences were identified and thymol, carvacrol, gama-tripinen were the main composition respectively. In another experiment Shahnazi et al. (2002) showed that the essences of 34 species of savory plant as the major components have thymol (25.25%), para-simen (21.44%), gama- terpinen (20%), carvacrol (9.48%), alfa- terpinen (7.94%) and Myrcene (3.58%).

Meat oxidative stability

Effects of savory essence, garlic powder and clover powder on the amount of dry matter, crude protein, and crude fat, water holding capacity and fat peroxidation in tissues of the breast at different times are demonstrated in tables. The results showed that the rate of dry matter, crude fat and water retention capacity is influenced by the trial treatments. The results showed that the level of 10 percent clover powder in compare to other treatments reduced water holding capacity and fat percentage of breast tissue. Water holding capacity in the meat due to two reasons is important: first, meat is sold on the basis of its weight hence any loss of water economically is undesirable. Secondly, the water holding capacity effects on fresh meat appearance [20]. The reduction in the WHC is because of acidity which results in loss of efficiency and the capability of connecting to the water, protein [21] and the development of muscle hardening after death [22] and leads to shortening the distance of the myoblast network, and therefore the necessary resources to move by water to the channel is provided [23]. Many of the compounds associated with metabolism, such as keratin and lactate, mitochondria, glycogen, ATP indirect water storage capacity is generally associated with meat. Regenstein, et al. suggested the potential connection of the water as the ability of the muscles protein in keeping the water which represents the highest amount of water existed in the muscle. About 88 to 95 percent of water presented in space between filaments of actin and myosin. While, only 5 to 12 percent of the water is located between myofibrils. factors such as pH, osmotic pressure, and length of sarcomere and improvement of muscle hardening after death with the affected put on the cellular and extracellular components, maintenance of water capacity was not affected. With increasing water in muscle, the tenderness, and the appearance of colored water in the muscles improved and lead to improved quality and economic value of meat. The major part of the water in the muscles located within the categories of myofibrils, fibrils and the muscle cell membrane (Sarcolemma), and between muscular cells and muscular masses. Some herbs like savory, especially because of polyphenol compounds particularly carvacrol having a decreased fat in the abdominal cavity, and reduced peroxidation of lipid, thereby increasing the potential of anti-oxidative muscle. Radical free oxygen due to the desire of a mix up for the reaction with important biomolecules such as amino acids, nucleic acids, fat and proteins, causing damage to membranes, enzymes, receptors and other cellular structures in different body tissues of chicken. Even though the body of the chicken with two types of mechanism and un-enzymatic procedures neutralize free radical but prevent the incidence of turning negative. Utilization of natural anti-oxidants in broiler diets causes reducing the peroxidation of lipids in feed and the texture of different body tissue [13]. The use of phytogenic leads to increasing the potential of muscle chest in chicken. The results are similar to quail and Turkey.

Table 3: Effect of additives of savory essence, clover powder and garlic powder on the amount of dry matter, crude protein, crude fat and water holding capacity.

Treatments	Dry matter	Crude protein	Crude fat	WHC
1	26.23^{ab}	86.11	8.48^{a}	317.07^{a}
2	26.82^{ab}	85.38	9.10^{a}	318.90^{a}
3	25.66^{ab}	86.11	7.87^{ab}	317.07^{a}
4	25.57^{b}	86.01	8.03^{ab}	316.73^{a}
5	26.38^{ab}	87.75	9.05^{a}	317.90^{a}
6	28.42^{a}	85.73	5.95^{ab}	316.82^{a}

7	26.48[ab]	86.28	5.01[b]	313.57[b]
MSE	1.37	1.92	1.62	1.48
P value	0.43	0.83	0.04	0.01

*Means values in a column with different superscripts are significantly different (P<0.05).

1=control without Additives, 2=400 mg/kg of essential savory, 3=500 mg/kg of essential savory, 4=4% garlic powder,5=5% garlic powder, 6=5% clover powder, 7=10% clover powder.

Characteristics of meat quality such as WHC, shear forces (SF), drip loss (DL), cook loss (CL), pH, shelf life (SL), the amount of collagen, protein break up ability (PBA), cohesiveness, and fat binding capacity (FBC) can be determined by industry rating at the time of production of tremendous value. Meat quality carries off physiological, structural and biological mechanism. The tenderness of meat is defined by amount or solubility of connective tissue, shortening each sarcomere during the progression of hardening of the muscle and proteolysis myofibril and myofibrils joined to proteins, after the death [25].

Effect of experimental treatments of savory essential oil, garlic powder and clover powder on fat peroxidation of breast tissue at different times showed statistical differences. With regards to the maintenance of breast meat at different times (0, 24 and 48 hours) in freezer, the amount of fat peroxidation showed statistical difference. The amount of acidity at different times for experimental treatments showed significant differences (P<0.05). The lowest amount of acidity belongs to treatment of 5% clover powder. The MDA production of breast meat in experimental treatments showed statistical differences (P<0.05). Maintenance at different times had significant impact on the amount of MDA breast meat (P<0.05).

Phytoestrogens are natural compounds that are found in many plants and have antioxidant and estrogenic attributes. Phytoestrogens are plant compounds that are similar in terms of structure and functioning which act like estrogens. Phytoestrogens of clover has antioxidant effects [26]. Physiological, structural and biological mechanisms carry off quality of the meat.

The production of MDA of breast meat at the zero time of incubation was significant (table 4). After 48 hours freezing, the concentration of MDA in breast meat which had received different levels of clover powder was less than the birds which were fed with essential savory and garlic powder (P<0.05). At the time 48 hours, the amount of MDA in breast meat of birds that in their rations received clover powder (0.14 and 0.09 and 400 mg/kg), was less than other treatments (P<0.05). Lipid peroxidation rate is one of the most destructive and harmful factors of meat quality. The lipid peroxidation rate in plasma and tissue is determined and evaluated by the use of MDA rate index, this unit is also called Tiobarbituric acid reaction store (TBARS). Oxidation can affect product quality due to loss of color, smell and taste which affect the life of the maintenance in the refrigerator.

In studies that compounds like β-cymene-2, 3-diol, thymol and carvacrol showed strong antioxidant properties, essence gives a good antioxidant role in systems of living organisms and act as effective free radicles in carcass. Production of MDA has carried the oxidative decay. Among the different methods for calculating the MDA, the method of TBA used highly for the calculation of the oxidation of lipids in many animal tissues. This experiment is based on the amount of pink color complex light color absorption produced from the reaction of molecule MDA with 2 molecules of TBA. This method is rapid, simple, and the most common form used for the calculation of the MDA in a sample of meat [27]. The essential oil and aquatic and alcoholic extract of savory can be used as a natural antioxidant source of cheap, available and potential of interest for food and drug purposes [28]

Shirali et al. reported that the amount of phenolic compounds of savory essential oils with regards to consistence of phenolic and flavonoid compounds as thymol and carvacrol cause antioxidant properties in this plant. This property leads to prevention of lipids oxidation, heart coronary and cancer diseases [9] **(Table 4)**.

Table 4: Effect of savory essence, clover powder and garlic powder additives on Fat peroxidation (pH, MDA, Free Nitrogen) in tissue of the breast meat of broilers at different times (hours).

	pH			Malonedialdehyde TBA			Free Nitrogen		
Items	0	24	48	0	24	48	0	24	48
1	5.85a	5.4bc	5.79bc	0.012bc	0.009b	0.09b	0.09b	00.24ab	67.26
2	5.77ab	5.41c	5.81ab	0.017bc	0.03ab	0.06b	0.06b	67.20b	67.19
3	5.67ab	5.58b	5.75bc	0.013abc	0.008b	1.59a	1.59a	33.29a	00. 20
4	5.80a	5.81a	5.91a	0.018a	0.05ab	0.13b	0.13b	33.18b	0.25
5	5.79a	5.85a	5.86ab	0.017ab	0.03ab	0.06b	0.06b	00.20b	33.23

6	5.67b	5.62b	5.68c	0.005d	0.06a	0.14b	0.14b	00.20b	50.21
7	5.78ab	5.82a	5.77bc	0.01cd	0.05ab	0.09b	0.09b	25.24ab	50.25
SEM	0.08	0.08	0.06	0.003	0.02	0.55	0.55	33.4	63.6
P value	0.15	0.001	0.01	0.004	0.12	11	11	9	77

*Means values in a column with different superscripts are significantly different (P<0.05).1=Control without Additives, 2=400 mg/kg of essential savory, 3=500 mg/kg of essential savory, 4=4% garlic powder,5=5% garlic powder, 6=5% clover powder, 7=10% clover powder.

The results showed that the rate of dry matter, crude fat and WHC is influenced by the experimental treatments. The results showed that the level of 10 percent clover powder reduced the percentage of WHC and fat breast tissue in relative to other treatments. WHC of meat is important for two reasons: firstly, meat is sold on the basis of its weight; hence any loss of water is in terms of adverse economic. Secondly, WHC effects on fresh meat appearance.

The results showed that the level of 500 mg/kg of savory essence and garlic powder level of 4% causes the maximum length of villi of small intestine in broilers. Likewise the lowest length of villi of small intestine belongs to clover powder treatment. The experimental diets which had levels of savory essence and garlic powder were compared to control group showed no statistical differences for length of villi of small intestine in broilers, but compared to the food rations containing clover powder demonstrated statistical differences. Other properties of the tissues of small intestines texture of broiler such as the width, depth, length, length to width and length to depth ratio of villi have significant differences.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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