Herbal plants as feed additives in broiler chicken diets

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ABSTRACT

This study evaluated the effect of some herbal plants as natural feed additives on broiler production parameters, carcass parts, and breast meat quality. A total of 150 broiler chicks, Cobb 500 hybrid, were allocated to five treatments with 30 broilers /group, for 42 days. The experimental treatments were: Control (C); a diet containing 5% alfalfa meal (A); a diet containing 5% alfalfa meal and 1% basil (AB); a diet containing 5% alfalfa and 1% thyme (AT) and a diet containing 5% alfalfa and 1% sage (AS). Bodyweight was significantly ($P<0.05$) affected in AS group compared with C and A groups. Feed conversion ratio was significantly higher only in A and AB groups. Experimental groups had significantly greater ($P<0.05$) gizzard weight. The total polyphenols content and the antioxidant capacity were significantly ($P<0.05$) higher in AB, AT, and AS groups versus the C group. The cholesterol concentration was significantly ($P<0.05$) decreased in AB, AT, and AS meat samples compared with C and A samples. Instrumental colour measurements indicated significant alterations ($P<0.05$) among the groups regarding the values of L^* , a^* , b^* , H° , C^* , and ΔE^* . The texture analysis showed that hardness, gumminess, and chewiness were significantly ($P<0.05$) higher in AB, AT, and AS groups than in C and A groups.

Keywords: additives, alfalfa, basil, thyme, sage, chickens, meat quality.

INTRODUCTION

Consumer demand for safe, natural and nutritious food of animal origin is a challenge for animal nutrition. Consumers' growing interest in bioactive components of natural origin has resulted in the use of herbal plants, as beneficial ingredients in the industries of feed and food production (Sacchetti et al., 2005), to replace the potentially harmful synthetic additives. These industries are currently searching for safe and efficient substances with

demonstrated benefits. There has been a great interest in many spices and herbs, as they are considered an unexploited reservoir of valuable substances that can accomplish such demands (Al-Shuwaili et al., 2015). In the literature, some evidence suggests that various herbal plants and their extracts have beneficial effects on animals' health, which improved food safety and quality, for the consumer (Valenzuela-Grijalva et al., 2017; Pandey et al., 2019). However, there is little evidence that including them as a mixture of plant-based foods would have beneficial growth effects in live birds.

Basil (*Ocimum basilicum* L.), thyme (*Thymus vulgaris* L.), and sage (*Salvia officinalis*) are all aromatic herbal plants, of the *Lamiaceae* family, used for their antimicrobial, antiseptic, antioxidant, antiviral, anti-inflammatory, and antibacterial properties (Turcu et al., 2020). They are mixtures of antioxidants and phenolic compounds, and for this reason, their addition to animal nutrition represents an interesting tool to provide supplements with biologically active compounds (Parham et al., 2020). The effect of basil in broiler chicken diets was reported to decrease mortality while increasing the dressing percentage and weight gain and improving feed conversion ratio (Jahejo et al., 2019). Ahangaran et al. (2019) reported that 0.5% and 1% of thyme in broiler chicken diets elevated body weight and decreased feed conversion ratio. Sage, is not widely used in its natural form, being more used as an essential oil extract, but the few current data showed that its usage in broilers diet had a beneficial effect on performances, health, and meat quality (Ibrahim et al., 2018). However, their effects on poultry performances have been inconsistent, but only a few studies reported negative effects or null effects. Although there's still a scarcity of data regarding the mechanism of action of varied herbal plants, they might be used as new-generation dietary ingredients for animal nutrition.

Alfalfa (*Medicago sativa* L.) is a common perennial feedstuff that is rich in protein and amino acids, as well as minerals and vitamins. It contains many vitamins, including A, D, E, K, C, B1, B2, B6, B12; minerals including P, Ca, K, Na, Cl, S, Mn, Co, Mg, Fe, Co, B, and Mo (Ponte et al., 2004; Jiang et al., 2012). It also contains lots of functional bioactive components, such as β -carotene, flavonoids, xanthophylls, and carotenoids that give carcasses their yellow colour (Zheng et al., 2019). Alfalfa meal is unique in its broad range of advantages. As a result, it's an excellent dietary feed for poultry with different purposes. For example, 3%, 6%, or 9% alfalfa meal improved carcass parts (Jiang et al., 2012). Previous studies have indicated that the addition of more than 5% alfalfa meal negatively influenced broiler chicken performances (Rivas Caceres et al., 2020). Little is known about the effect of using alfalfa meal together with the above-mentioned herbal plants in broilers nutrition.

In this context, the present study aimed to determine the effect of alfalfa meal used together with basil, thyme, or sage herbal plants as natural feed

additives included in broiler chickens' diet on performances, organs development, and breast muscle quality.

MATERIALS AND METHODS

The Ethical Committee of the institute, approved the experimental procedures according to the Romanian legislation (Law 206/2004, ordinance 28/31.08.2011, Law 43/ 11.04.2014, Directive 2010/63/EU).

Plant materials

The herbal plant material used in this study (basil, thyme, and sage) was purchased in ready to use form from Plafar, a local Agency for Medicinal plants from Bucharest, Romania, which works under the coordination of the Health Ministry, having as main activity objective conditioning, primary processing of raw materials and capitalization in the form of pharmaceutical products, but not only. Alfalfa was purchased in the pelleted form a local farmer. The leaves of the herbal plants and alfalfa were powdered in a hammer mill with a 1-mm screen. The powder was then stored at room temperature in paper bags until used in compound feeds.

Animals, Diets, and Housing

A total of 150-day-old broiler chickens, Cobb 500 hybrid, were purchased from a local hatchery. The broiler chicks were housed in an experimental hall equipped with three-tiered Big Dutchman digestibility cages (cage dimensions 65 × 75 × 45 cm). The temperature inside the experimental hall was maintained at 33°C for the first three days, and after was reduced gradually to 24°C until the end of the experiment. The light regimen was set according to the Cobb 500 broiler management guide. The broiler chickens were fed during the starter period (1-10 days) with a standard feed based on corn, soybean meal, and corn gluten, having 21.41% crude protein and 2975.72 kcal /kg metabolizable energy. At 10 days of age, the chickens were weighed individually and randomly assigned to five groups (C, A, AB, AT, AS) with 30 broilers in each group of mixed-sex, established at the beginning of the experimental period. During the grower phase (11-28 days), finisher I phase (29-35 days) and finisher II phase (36-42 days) the given dietary treatments were as follows: control diet (C); a diet containing 5% alfalfa meal (A); a diet containing 5% alfalfa meal and 1% basil (AB); a diet containing 5% alfalfa and 1% thyme (AT) and a diet containing 5% alfalfa and 1% sage (AS). All diets were composed to meet the requirements suggested by the Cobb 500 Management Breeding Guide. The ingredients and nutritional composition of the diets are shown in Tables 1, 2, and 3.

Throughout the experimental period they had ad libitum access to the feeds and water, without any medical care program.

Table 1. Ingredients of the grower diets, % as fed-basis

Ingredients	C	A	AB	AT	AS
	Grower (11-28 days)				
Corn	44.94	45.00	44.86	44.87	45.00
Soy meal	23.31	24.75	24.52	24.70	24.72
Corn gluten	5.00	5.00	5.00	5.00	5.00
Wheat	19.23	11.87	11.05	10.81	10.55
Vegetal oil	2.72	4.32	4.40	4.45	4.56
Alfalfa meal	-	5.00	5.00	5.00	5.00
Basil	-	-	1.00	-	-
Thyme	-	-	-	1.00	-
Sage	-	-	-	-	1.00
Acidifying	-	0.10	0.10	0.10	0.10
L – Lysine - HCl	0.38	0.10	0.10	0.10	0.10
DL-Methionine	0.29	0.18	0.17	0.18	0.18
L-Threonine	0.10	0.10	0.10	0.10	0.10
Choline	0.04	0.04	0.04	0.04	0.04
Ca. carbonate	1.32	0.96	0.97	0.96	0.96
Monocalcium Phosphate	1.31	1.33	1.34	1.34	1.34
Salt	0.36	0.35	0.35	0.35	0.35
Vitamin-Mineral premix*	1.00	1.00	1.00	1.00	1.00
Total ingredients, %	100	100	100	100	100
Calculated nutrients					
Metabolizable Energy, kcal/kg	3025.4	3025.53	3079.17	3025.11	3025.96
Crude Protein, %	19.46	19.71	19.72	19.69	19.66
Ether Extract, %	4.78	6.40	6.47	6.54	6.65
Ash, %	2.55	3.20	3.17	3.18	3.18
Crude Fiber, %	3.45	4.43	4.51	4.56	4.68
Calcium, %	0.84	0.84	0.84	0.84	0.84
Available Phosphorus, %	0.42	0.42	0.42	0.42	0.42
Lysine, %	1.21	1.22	1.22	1.22	1.22
Methionine + Cysteine, %	0.93	0.94	0.94	0.55	0.94
Threonine, %	0.81	0.81	0.81	0.81	0.81
Tryptophan, %	0.19	0.19	0.19	0.19	0.19
Determined nutrients					
Polyphenol's content, mg GAE/g	2.36	2.43	2.58	2.91	3.17
Antioxidant capacity, mM Trolox	14.81	14.77	16.97	19.27	19.15
Lutein and Zeaxanthin, mg/g	8.53	9.87	10.88	11.13	11.99
Vitamin E, mg/g	42.00	69.74	56.08	82.75	50.38

The components of 1% premix are presented elsewhere (Vlaicu et al. 2020a).

Table 2. Ingredients of the early finisher diets, % as fed-basis

Ingredients	C	A	AB	AT	AS
	Finisher I (29-35 days)				
Corn	45.00	44.10	44.90	44.89	44.90
Soy meal	20.30	20.77	20.48	20.79	21.07
Corn gluten	5.00	5.00	5.00	5.00	5.00
Wheat	21.79	15.55	14.76	14.39	13.99
Vegetal oil	3.52	5.00	5.08	5.15	5.27
Alfalfa meal	-	5.00	5.00	5.00	5.00
Basil	-	-	1.00	-	-
Thyme	-	-	-	1.00	-
Sage	-	-	-	-	1.00
Acidifying	-	0.10	0.10	0.10	0.10
L - Lysine - HCl	0.33	0.08	0.08	0.08	0.07
DL-Methionine	0.26	0.16	0.15	0.15	0.15
L-Threonine	0.10	0.08	0.08	0.08	0.08
Choline	-	-	-	-	-
Ca. carbonate	1.20	0.85	0.85	0.85	0.85
Monocalcium Phosphate	1.14	1.15	1.16	1.16	1.16
Salt	0.36	0.35	0.36	0.36	0.36
Vitamin-Mineral premix*	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Calculated nutrients					
Metabolizable Energy, kcal/kg	3100.97	3100.12	3100.76	3100.70	3100.55
Crude Protein, %	18.50	18.50	18.50	18.50	18.50
Ether Extract, %	5.60	7.11	7.18	7.25	7.38
Ash, %	2.40	2.99	2.96	2.98	2.99
Crude Fiber, %	3.31	4.25	4.33	4.39	4.50
Calcium, %	0.76	0.76	0.76	0.76	0.76
Available Phosphorus, %	0.38	0.38	0.38	0.38	0.38
Lysine, %	1.10	1.11	1.11	1.11	1.11
Methionine + Cysteine, %	0.88	0.88	0.88	0.88	0.88
Threonine, %	0.77	0.73	0.73	0.73	0.80
Tryptophan, %	0.18	0.18	0.18	0.18	0.73
Determined nutrients					
Polyphenol's content, mg GAE/g	2.28	2.37	2.56	3.17	2.62
Antioxidant capacity, mM Trolox	17.24	17.33	18.81	23.71	18.84
Lutein and Zeaxanthin, mg/g	9.31	10.52	11.34	11.13	12.38
Vitamin E, mg/g	43.13	55.54	57.23	57.05	56.68

The components of 1% premix are presented elsewhere (Vlaicu et al. 2020).

Table 3. Ingredients of the late finisher diets, % as fed-basis

Ingredients	C	A	AB	AT	AS
	Finisher II (36-42 days)				
Corn	44.95	44.86	44.86	44.85	44.85
Soy meal	16.94	17.33	17.03	17.34	17.62
Corn gluten	5.00	5.00	5.00	5.00	5.00
Wheat	24.70	18.51	17.73	17.35	16.96
Vegetal oil	3.99	5.46	5.53	5.61	5.73
Alfalfa meal	-	5.00	5.00	5.00	5.00
Basil	-	-	1.00	-	-
Thyme	-	-	-	1.00	-
Sage	-	-	-	-	1.00
Acidifying	-	0.10	0.10	0.10	0.10
L - Lysine -HCl	0.36	0.11	0.11	0.11	0.10
DL-Methionine	0.25	0.14	0.14	0.14	0.14
L-Threonine	0.08	0.09	0.09	0.09	0.09
Choline	0.04	0.04	0.04	0.04	0.04
Ca. carbonate	1.22	0.87	0.87	0.87	0.87
Monocalcium Phosphate	1.14	1.16	1.17	1.17	1.17
Salt	0.33	0.33	0.33	0.33	0.33
Vitamin-Mineral premix*	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Calculated nutrients					
Metabolizable Energy, kcal/kg	3207.50	3150.63	3150.28	3150.21	3150.06
Crude Protein, %	17.50	17.50	17.50	17.50	17.50
Ether Extract, %	6.08	7.58	7.65	7.73	7.85
Ash, %	2.22	2.81	2.78	2.80	2.81
Crude Fiber, %	3.15	4.08	4.16	4.22	4.34
Calcium, %	0.76	0.76	0.76	0.76	0.76
Available Phosphorus, %	1.05	0.38	0.38	0.38	0.38
Lysine, %	0.53	1.05	1.05	1.05	1.05
Methionine +Cysteine, %	0.84	0.84	0.84	0.84	0.84
Threonine, %	0.70	0.70	0.70	0.70	0.70
Tryptophan, %	0.17	0.16	0.16	0.16	0.16
Determined nutrients					
Polyphenol's content, mg GAE/g	2.06	2.07	2.27	2.66	2.26
Antioxidant capacity, mM Trolox	17.56	17.57	18.16	19.70	18.52
Lutein and Zeaxanthin, mg/g	7.96	8.41	11.59	12.78	12.38
Vitamin E, mg/g	43.12	56.10	70.56	57.19	56.68

The components of 1% premix are presented elsewhere (Vlaicu et al. 2020).

Growth Performances and Carcass Parts

The production performances were calculated at the end of the trial (42 days). Then, 30 broiler chickens (6 chickens /group) with homogenous weights were selected to be slaughtered as presented previously (Vlaicu et al., 2020a). The defeathered carcass, without head and feet, was eviscerated manually and weighed as carcass weight. After dissection and evisceration, organs measurements were performed (breast muscle, thigh muscle, liver, gizzard, and heart), and the samples of breast were collected and sampled to determine the proximate chemical composition, antioxidant compounds, cholesterol concentration, colour, and texture profile. Until chemical analyses were performed the samples were stored in plastic zipped bags at -20°C.

Primary Chemical Analysis

The primary chemical composition analyses were determined on samples dried at 65°C, according to the Association of Official Analytical Chemists (AOAC, 1990) and complied with Regulation (CE) no 152 /2009. The crude protein (CP) was determined by the Kjeldahl method according to standard SR EN ISO 5983-2:2009 (Kjeltec auto 1030 - Tecator Instruments, Hoganas, Sweden). Crude fat (EE) was determined by extraction in organic solvents - the method complies with standard SR EN ISO 6492:2001 (Soxtec 2055 - Foss Tecator, Sweden). Crude fibre (CF) was determined by the method with intermediary filtration, according to standard SR EN ISO 6865:2002 (Fibertec 2010 System - Foss Tecator, Sweden).

Antioxidant Compounds

The total antioxidant capacity of the samples was measured by the phosphomolybdenum method (Untea et al., 2020) with absorbance recorded at 695 nm. The results were expressed as mM Trolox. Folin-Ciocalteu's method was used to measure spectrophotometrically the total polyphenols content and the results were expressed as milligrams gallic acid equivalents per gram (mg GAE/g).

Lutein and zeaxanthine were determined using high-performance liquid chromatography (Perkin Elmer 200 series, Shelton, CT, USA) with a UV detector (445 nm). Vitamin E was determined according to the EU Regulation EC 152/2009, and it used a high-performance liquid chromatograph (HPLC Finnigan Surveyor Plus) and a PDA-UV detector (wavelength of 292 nm), as described by Untea et al. (2020).

Cholesterol Determination

Cholesterol concentration of the breast meat samples was determined by gas chromatography, (Perkin Elmer-Clarus 500, GC) according to AOAC International 1996, as described elsewhere (Turcu et al., 2019).

Instrumental Colour Determination

A Konica Minolta CR-400 (Holdings Inc., Tokyo, Japan) colourimeter performed chicken breast meat colour analyses. The colourimeter was calibrated using the standard white ceramic reference (illuminate C). The average of 6 measurements /sample (36 measurements/group) at different spots on the breast muscle meat was recorded for lightness (L^*), redness (a^*), and yellowness (b^*) according to the CIE Lab (CIE, *Commission Internationale de l'Eclairage*, Vienna, Austria) trichromatic system. The readings were automatically used to characterize the surface colour parameters ΔL , Δa , and Δb . Breast meat colour measurements were taken in a clean area without colour defects that may affect a uniform colour reading. To evaluate the colour changes of the breast meat colour in time, readings were taken over 24h, 48h, and 72h respectively, at 4°C ageing time. The obtained results were used to calculate psychometric colour terms involving hue angle (H°), chroma (C^*) and colour difference over time (ΔE), with appropriate formulas:

$$\text{Hue angle} \quad H^\circ = \arctan^{-1}(b/a) \quad (1)$$

$$\text{Chroma} \quad C^* = \sqrt{a^2 + b^2} \quad (2)$$

$$\text{Colour difference over time} \quad \Delta E^* = \sqrt{L^2 + a^2 + b^2} \quad (3)$$

On a scale of 0 to 100, L^* represent dark to light, on a scale of -60 to +60 a^* represents green to red, and b^* represents blue to yellow. The closest values to 0° or 360° are considered preferable to consumers for H^* (CIE system).

Texture Profile Analysis (TPA)

The TPA of breast muscle meat samples was determined with a CT3 Texture Analyzer (AMETEK Brookfield, DKSH Australia Pty Ltd.) and a software package. The TPA parameters of chicken breast meat was evaluated after the previously described method (Vlaicu et al., 2020b), with slight modification, adapted to CT3. For that, two cylinders of 10 mm height and 10 mm diameter were prepared from the six meat samples from each group. A double compression cycle test was performed with a glass cylinder of 20 mm diameter up to 70% compression of the original portion height. A force–time curve was obtained with a crosshead speed of 2 mm/second. The load cell was 10 kg, and the time interval between compression cycles was 5 seconds. The data obtained (24 values in each group) from the TPA curve were used for the calculation of textural parameters. The CT3 Texture Analyzer can calculate, through compression and tensile data, several physical properties. These properties like tenderness (hardness), adhesiveness, resilience, springiness, cohesiveness, chewiness, and gumminess, have been correlated to consumer sensory evaluation of food products.

Statistical Analysis

The data were analysed using variance analysis (ANOVA) in Stat View for Windows (SAS, version 6.0). One-way ANOVA was used to compare broiler performance and breast muscle meat quality. The difference between the means was significant at $P < 0.05$. Significant differences among treatment means were evaluated using Tukey's multiple range tests.

RESULTS AND DISCUSSION

Effect of Dietary Treatments on Growth Performances

The effects of dietary supplementation with alfalfa meal and different herbal plants on body weight (BW), average daily weight gain (ADWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) of Cobb 500 broiler chickens are presented in Table 4. At 35 days, BW was significantly ($P < 0.05$) higher in the C group compared with all experimental groups, while at 42 days BW was significantly higher ($P < 0.05$) only in C and A groups compared with AS group. A similar effect was also noted for ADWG at the end of the trial. ADFI was significantly higher in both finishing phases in the C group compared with AS group. FCR was significantly ($P < 0.05$) higher only in A and AB groups compared with the C group for the overall experimental period. No mortality was recorded during the experimental period. The supplementation with herbal plants revealed that only sage produced significant ($P < 0.05$) alterations on broiler performances, while the other treatments had close values to the C group. This effect in AS group could be provoked by the phenolic compounds from mono-di and triterpenoids such as the presence of high saponins content from both alfalfa and sage suppressing feed consumption because of the bitter taste, which affects the broiler response, in terms of BW. The reducing growth effect of sage supplementation was also observed by Farhadi et al., (2020) when 1.20% sage was used in broiler's diets. While some studies reported improvements on BW and ADFI (Lau and King, 2003), other studies reported opposite effects, probably due to high inclusion level of alfalfa combined with the herbal plants, containing higher levels of crude fibre, which could cause nutrient imbalances and inhibit metabolism, thus negatively affecting growth performance (Righi et al., 2021). Another explanation could be that plants with feed additive properties decrease the pathogenic microorganisms, stimulating the immune system, and therefore instead of growing muscles, strengthen the immune system of broilers. Recently it was resumed that, the supplementation with alfalfa meal 4%, 6%, and 7.3% in the diet of chickens showed lower ($P < 0.05$) BW, ADFI, and FCR, while increasing the proliferation of beneficial bacteria and inhibiting potential pathogens (Yue et al., 2019; Gulizia et al., 2020; Pliego et al., 2020). Moreover, it was reported that the difference between feeding single herbal plants versus herbal mixture might be due to some antimicrobial

components present in the ingredients of the herbal plants used in the present study (Saleh et al., 2018). This could explain why various studies have reported that herbal plants and leaf meals used alone or in different combinations had positive, negative, or no effect effects at all on broilers performances.

Table 4. Effects of dietary treatments on broiler chickens' performance (10-42 days)

Item	C	A	AB	AT	AS	SEM	P
Bodyweight (BW), g							
10 d	482.52	482.14	482.14	482.73	488.61	4.796	0.7484
28 d	1597.82	1521.32	1523.16	1509.47	1521.84	19.83	0.2009
35 d	2249.74 ^a	2157.89 ^b	2169.21 ^b	2143.42 ^b	2143.68 ^b	12.03	0.0399
42 d	3028.67 ^a	3014.67 ^a	2960.00 ^{ab}	2924.33 ^{ab}	2881.33 ^b	34.53	0.0291
Average daily weight gain (ADWG), g							
10 - 28 d	79.59	74.23	74.36	73.34	73.80	1.048	0.1191
28 - 35 d	99.72 ^a	90.94 ^b	83.72 ^b	90.56 ^b	88.83 ^{ab}	1.360	0.0019
35 - 42 d	106.95 ^a	103.71 ^{ab}	103.62 ^{ab}	103.19 ^{ab}	99.62 ^b	2.689	0.0105
10 - 42 d	91.28 ^a	89.92 ^a	87.53 ^b	85.66 ^b	84.09 ^b	1.100	0.0123
Average daily feed intake (ADFI), g/chick							
10 - 28 d	110.62	103.43	105.35	107.92	104.39	2.746	0.9047
28 - 35 d	167.64 ^a	161.67 ^{ab}	160.48 ^{ab}	163.73 ^{ab}	157.48 ^b	2.125	0.0336
35 - 42 d	199.59 ^a	184.27 ^b	193.60 ^{ab}	185.03 ^b	186.85 ^b	3.104	0.0299
10 - 42 d	150.18	141.05	144.37	144.06	141.58	3.235	0.9117
Feed conversion ratio (FCR), kg feed/chick							
10 - 28 d	1.20	1.18	1.22	1.20	1.20	0.017	0.2279
28 - 35 d	1.50 ^b	1.59 ^{ab}	1.62 ^a	1.61 ^a	1.57 ^{ab}	0.031	0.0181
35 - 42 d	1.75 ^b	1.79 ^{ab}	1.82 ^a	1.80 ^a	1.79 ^{ab}	0.045	0.0432
10 - 42 d	1.76 ^b	1.80 ^a	1.81 ^a	1.79 ^{ab}	1.78 ^{ab}	0.027	0.0439

^{a-b} Means in the same row with different superscript letters are significantly different by Tukey's multiple comparison method ($P < 0.05$). C- control diet; A - a diet containing 5% alfalfa meal; AB - a diet containing 5% alfalfa meal and 1% basil; AT - a diet containing 5% alfalfa and 1% thyme; AS - diet containing 5% alfalfa and 1% sage.

Effect of Dietary Treatments on Carcass Characteristics

Weights of the eviscerated carcass, thigh muscle, breast muscle, liver, gizzard, and heart are presented in Table 5. Thigh muscle weight was significantly ($P < 0.0494$) higher in A and AS groups compared with the C group, while gizzard weight was significantly increased ($P < 0.0305$) in all experimental groups fed diets supplemented with alfalfa meal and herbal plants. Breast muscle weight was significantly ($P < 0.0168$) higher only in the C group, compared with the A and AB groups. No significant differences ($P > 0.05$) were recorded for liver and heart weight. The significant effect observed for breast muscle weight corresponds to final BW in the C group. Thus, birds fed the C diet had higher BW and, as it was expected the breast muscle meat weighed more than birds from A, AB, AT, and AS diets at 42 days of age. However, the significantly ($P < 0.05$) higher thigh muscle weight in A

and AS groups is not correlated with the total BW of chicken broilers. Feeding diets supplemented with alfalfa and herbal plants led to significant modifications of the gizzard compared with the C group. Larger gizzard sizes are beneficial to improve gut motility and stimulate the secretion of pancreatic enzymes (Shirzadegan and Taheri, 2017). Previously we obtained significantly ($p < 0.05$) higher gizzard in broiler fed oregano plant and oregano essential oil (Vlaicu et al., 2020c). Similarly, Ashour et al. (2020), reported that the highest ($P < 0.05$) weight of gizzard and heart was in broilers fed with 2% herbal plant mixture and C group, without any effect on other organs. On the other hand, Abou-Elkhair et al. (2014) and Amouzmehr et al. (2012) reported no significant effect in the gizzard, heart, and liver across the groups fed different herbal plants and mixtures. This observation suggests that different herbal plants have varying effects on the carcass development of broiler chickens. We can assume that alfalfa meal had a greater influence on gizzard size than herbal plants added, because of its high content of crude fibre. However, the effect of alfalfa meal on the carcass development of broilers has been scarcely reported, even though these measurements are important parameters that reflect the growth development of broilers.

Table 5. The influence of herbal plants on carcass development

Item	C	A	AB	AT	AS	SEM	P
Eviscerated carcass, g	2288	2343	2280	2370	2353	18.09	0.4288
Thigh muscle, g	499.7 ^b	554.1 ^a	524.4 ^{ab}	527.2 ^{ab}	560.5 ^a	7.783	0.0494
Breast muscle, g	631.1 ^a	514.1 ^b	546.6 ^b	577.6 ^{ab}	562.1 ^{ab}	13.95	0.0168
Liver, g	65.08	66.26	60.38	63.86	64.30	1.653	0.8598
Gizzard, g	33.90 ^b	38.19 ^a	37.38 ^a	36.25 ^a	36.45 ^a	0.955	0.0305
Heart, g	15.36	16.46	16.04	16.06	16.13	0.399	0.8951

^{a-b} Means in the same row with different superscript letters are significantly different by Tukey's multiple comparison method ($P < 0.05$). C- control diet; A - a diet containing 5% alfalfa meal; AB - a diet containing 5% alfalfa meal and 1% basil; AT - a diet containing 5% alfalfa and 1% thyme; AS - diet containing 5% alfalfa and 1% sage.

Effect of Dietary Treatments on Nutritional Composition of Breast Muscle

Results obtained from the nutritional composition of breast muscle samples are presented in Table 6. The lowest crude protein concentration ($P < 0.0048$) was determined in the A sample compared with the other samples. Interesting is that the same treatment A and AB had significantly ($P < 0.0320$) higher concentrations of crude fat, compared with C samples. This result is beneficial because high protein content makes chicken meat an ideal foodstuff for all consumers who need high-quality and easily degradable protein. In contrast, Souza et al. (2011) reported that protein concentration of chicken breast muscle meat ranged from 22.48 to 22.61% without significant differences, while Mohammed (2013) reported values ranging from 20.70 to 32.10% when different herbal plants were fed. On the other hand, Baghban

Kanani et al. (2017) reported no effect of turmeric or cinnamon on the nutritional composition of thigh meat of broilers. Supplementation of diverse feed additives with high antioxidant possessions to chicken feed influences their deposition in poultry tissue, thus enabling the production of food with various nutritional constituents.

Table 6. Nutritional composition of broiler chickens breast muscle

Item	C	A	AB	AT	AS	SEM	P
Dry matter, %	26.46 ^b	25.65 ^b	28.51 ^a	28.41 ^a	26.65	0.324	0.0081
Crude protein, %	22.73 ^{ab}	21.15 ^b	24.09 ^a	23.78 ^a	23.42 ^a	0.294	0.0048
Crude fat, %	2.05 ^b	2.52 ^a	2.50 ^a	2.47 ^a	2.18 ^b	0.073	0.0320
Ash, %	1.25	1.28	1.30	1.31	1.29	0.015	0.0534

^{a-b} Means in the same row with different superscript letters are significantly different by Tukey's multiple comparison method ($P < 0.05$). C- control diet; A - a diet containing 5% alfalfa meal; AB - a diet containing 5% alfalfa meal and 1% basil; AT - a diet containing 5% alfalfa and 1% thyme; AS - diet containing 5% alfalfa and 1% sage.

Effect of Dietary Treatments on Antioxidant Compounds and Cholesterol in Meat

As shown in Table 7, supplementation with basil, thyme, and sage significantly ($P < 0.0413$) increased the total phenols content of breast meat in AB, AT, and AS groups, compared with C and A groups. Also, the antioxidant capacity was significantly ($P < 0.0261$) greater in AB, AT, and AS groups, compared with the C group, but without a significant effect on the A group. These results display that the antioxidative action in the light-colored meat of broiler chickens can be elevated by the tested feed additives. Further, the cholesterol concentration in the fat from the breast meat samples was significantly ($P < 0.0009$) lower in AB, AT, and AS experimental groups compared with C and A groups (Table 7). Some natural feed additives contain various compounds, for the most part polyphenols that have antioxidant activities, so they can enhance the quality of broiler meat. Phenolic compounds present in natural feed additives are absorbed in the circulatory system of chickens after ingestion and have significant antioxidant activities in chicken meat. After that, they react with lipid and hydroxyl radicals and convert them into more resistant meat products. The antioxidant properties of many herbal plants studied have been described to present high antioxidant effects. Some authors (Franz et al., 2010; Loetscher et al., 2013; Ahmed et al., 2018), reported that rosemary, sage, oregano, and thyme extracts have the potential to improve the oxidative stability of broiler meat due to their antioxidant properties, by interrupting free-radical chains resulting in a product with higher oxidative stability.

The significant decrease of cholesterol concentration in breast meat could be caused by the synergistic effects of dietary treatments used. Abdulkarimi et al. (2011) reported that different levels of thyme significantly lowered the

cholesterol concentration. Other spices including garlic, black pepper, and hot red pepper were reported to have a positive influence on cholesterol concentrations and lipid oxidation process in chicken meat (Puvača et al., 2013). We believe that the combination of alfalfa with basil, thyme, and sage caused the cholesterol decrement because combined they present higher concentrations of some components such as saponins, polysaccharides, and flavonoids. The flavonoids, exhibit differing biological activities in a way that antioxidant activity leads to modulating the cholesterol concentration in meat. A reduction of cholesterol concentration was also reported by Ouyang et al. (2016), indicating that the alfalfa might play an important role in adjusting lipid metabolism. However, few studies were done on the effects of herbal plants mixture on the cholesterol concentration in broilers meat. Although a possible synergism between antioxidant activities associated with lower cholesterol concentration, might cause these observations.

Table 7. Effect of dietary treatments on breast muscle antioxidants and cholesterol

Item	C	A	AB	AT	AS	SEM	P
Antioxidant compounds							
Polyphenols, mg GAE/g	1.033 ^b	1.035 ^b	1.122 ^a	1.134 ^a	1.138 ^a	0.037	0.0413
Antioxidant capacity, mM Trolox	1.635 ^b	1.718 ^{ab}	1.737 ^a	1.790 ^a	1.830 ^a	0.043	0.0261
Cholesterol concentration							
cholesterol in meat mg/100g	60.65 ^a	60.66 ^a	55.93 ^b	54.42 ^b	51.02 ^b	0.962	0.0009

^{a-b} Means in the same row with different superscript letters are significantly different by Tukey's multiple comparison method ($P < 0.05$). C- control diet; A - a diet containing 5% alfalfa meal; AB - a diet containing 5% alfalfa meal and 1% basil; AT - a diet containing 5% alfalfa and 1% thyme; AS - diet containing 5% alfalfa and 1% sage.

Effect of Dietary Treatments on Breast Meat Instrumental Colour

In Table 8 we present the effect of dietary herbal plants effect on the colour of breast muscle samples determined after 24h, 48h, and 72h of storage at 4°C. Feeding broiler chicken with AT and AS diets, significantly ($P < 0.0001$) influence the value of the L^* parameter after 24h compared with C, A, and AB groups. In contrast, the value of a^* was significantly lower ($P < 0.0371$) in the AB group, compared with C, A, and AT samples. The highest yellowness (b^*) was determined in samples from AS group, followed by the AB group, both being significantly ($P < 0.0001$) higher compared with C and A groups. ΔL^* and Δa were without significant effect among the groups, but the highest Δb was determined for AS samples, compared with all groups. C^* and ΔE^* , values were significantly ($P < 0.05$) higher in AS samples compared with the other groups. After 48h, AT and AS treatments maintained the highest ($P < 0.001$) lightness (L^*), while after 72h only AT samples registered higher ($P < 0.0435$) values for

the L^* parameter compared with AB samples. The saturation index in green/red (a^*) registered significant differences among the groups after 48h and 72h of storage. For the b^* parameter, after 72h, AS breast muscle samples had the highest value, followed by AT, both being significantly higher ($P<0.05$) compared with C, A, and AB samples. Chroma (C^*) parameter was significantly higher ($P<0.05$) in AS group after 48h and 72h, while the ΔE^* parameter was significantly ($P<0.05$) higher only after 72h.

The appearance, especially the colour of chicken meat is the main factor that influences the purchasing decisions of customers. For this reason, colour variation is a major problem in chicken meat marketing because the consumers are more attracted and sensitive to colour variations which led to a tendency to reject meat parts with noticeable colour differences. Although we observed higher lightness (L^*) in the meat of experimental groups, Baghban Kanani et al. (2017), reported lowered lightness by feeding turmeric and cinnamon as dietary feed additives in broiler diets. Others reported no effect of rosemary and sage essential oil on colour parameters (Chen et al., 2008). In another study, green tea extract increased the redness (a^*) and yellowness (b^*) parameters in meat. We assume that these discrepancies may be due to the different plants of the above-mentioned researches and those used in our experiment. Some research reports have different opinions when it comes to the influence of age on meat colour (Smith et al., 2002). Factors like age, genetic factors, diets, muscle parts (breast, thigh, leg) led to significant variations in meat colour appearance during processing (Petracci et al., 2004). It was also suggested that the differences occurred in broiler chicken meat are caused by the myoglobin content, but it was found that only thigh meat is affected due to the high proportion of red muscle fibres, while the breast is generally entirely composed of white fibres (Qamar et al., 2019). Our results regarding the positive influence of dietary treatments on meat colour could be caused by the higher concentration of xanthophylls like lutein and zeaxanthin, determined in the chemical composition of the compound feeds.

Effect of Dietary Treatments on Texture Parameters

The texture of meat is an important factor in the eating quality of broiler chickens, associated with consumer satisfaction. The supplementation of dietary treatments with alfalfa meal and herbal plants had a significant effect ($P<0.05$) on breast muscle hardness, gumminess, and chewiness, in all groups supplemented with herbal plants, except group A compared with the C group (Table 9). The hardness of the samples from the AB, AT, and AS groups was double that of C samples. The same trend was registered for gumminess and chewiness. A similar effect of significantly higher hardness, gumminess, and chewiness in commercial broiler breast meat was also reported by Devatkal et al. (2019) without any effect on other textural parameters.

Table 8. Effect of dietary treatments on broiler chickens breast muscle colour

Item	C	A	AB	AT	AS	SEM	P
24 h							
L*	39.99 ^{cb}	40.31 ^{cb}	40.58 ^b	42.53 ^a	43.33 ^a	0.285	<0.0001
a*	-1.12 ^a	-0.99 ^a	-0.51 ^b	-1.01 ^a	-0.78	0.069	0.0371
b*	3.68 ^c	3.42 ^c	4.10 ^b	3.86 ^{bc}	4.96 ^a	0.099	<0.0001
ΔL^*	0.309	0.368	0.721	0.354	0.598	0.064	0.1829
Δa	0.327	0.368	0.721	0.354	0.598	0.064	0.2101
Δb	0.748 ^{bc}	0.517 ^c	1.197 ^b	0.885 ^{bc}	2.146 ^a	0.102	<0.0001
H°	89.20	89.22	89.50	89.37	89.29	0.037	0.0687
C*	3.88 ^{bc}	3.58 ^c	4.26 ^b	4.04 ^{bc}	5.05 ^a	0.092	<0.0001
ΔE^*	0.66 ^c	0.97 ^{bc}	1.26 ^b	1.22 ^b	1.88 ^a	0.072	<0.0001
48 h							
L*	39.42 ^c	41.43 ^{ab}	40.22 ^{bc}	42.71 ^a	42.39 ^a	0.258	<0.0001
a*	-1.247 ^{ab}	-0.980 ^{bc}	-0.594 ^d	-0.893 ^c	-1.301 ^{ab}	0.053	<0.0001
b*	3.192	3.258	3.216	3.683	3.902	0.109	0.1274
ΔL^*	-2.679 ^b	-0.670 ^{ab}	-1.886 ^b	0.599 ^a	0.248 ^a	0.258	<0.0001
Δa	0.285 ^c	0.453 ^{bc}	0.996 ^a	0.631 ^b	0.290 ^c	0.057	<0.0001
Δb	0.261 ^b	0.222 ^b	0.295	0.785	0.966 ^a	0.109	0.1026
H°	89.16	89.19	89.19	89.23	89.17	0.136	0.5822
C*	3.45 ^b	3.43 ^b	3.34 ^b	3.83	4.17 ^a	0.092	0.0193
ΔE^*	1.83	2.10	2.21	2.42	2.35	0.119	0.6190
72 h							
L*	40.26	40.62	40.05 ^b	41.40 ^a	41.12	0.205	0.0435
a*	-1.086 ^a	-1.377 ^b	-0.775 ^c	-1.337 ^b	-0.957 ^c	0.051	0.0002
b*	3.119 ^c	3.678 ^c	3.616 ^c	4.142 ^b	4.892 ^a	0.116	<0.0001
ΔL^*	0.253	0.578	0.090 ^b	1.437 ^a	1.101	0.214	0.2174
Δa	-0.604 ^a	-0.828 ^a	-0.226 ^b	-0.787 ^a	-0.432 ^b	0.051	0.0002
Δb	-0.428	0.075	-0.308	1.202	1.497	0.131	<0.0001
H°	89.19	89.15	89.22	89.20	89.23	0.012	0.2327
C*	3.32 ^c	3.96 ^{bc}	3.75 ^c	4.36 ^b	5.08 ^a	0.096	<0.0001
ΔE^*	0.81 ^c	1.48 ^b	1.13 ^{bc}	1.60 ^b	2.21 ^a	0.682	0.0006

^{a-c} Means in the same row with different superscript letters are significantly different by Tukey's multiple comparison method ($P < 0.05$). C- control diet; A - a diet containing 5% alfalfa meal; AB - a diet containing 5% alfalfa meal and 1% basil; AT - a diet containing 5% alfalfa and 1% thyme; AS - diet containing 5% alfalfa and 1% sage.

Also, in a recent study, it was reported that severe woody breast samples were harder and gummier than normal samples. In the same study, moderate woody samples were gummier than normal samples (Jarvis et al., 2020). Jayasena et al. (2013) reported that texture parameters of broilers fed different herbal plant mixtures were influenced by age and genotype. This variability in our results was probably caused by the difference in total collagen content between the muscles which have the potential to influence the texture and hardness characteristics of the meat. These parameters could

vary from animal to animal, and further studies are needed to examine the recovering rate from *rigor mortis*, after slaughter.

Table 9. Effect of dietary treatments on broiler chickens breast muscle texture

Specification	C	A	AB	AT	AS	SEM	P
Hardness Cycle 1, g	1561.3 ^c	2262.9 ^{bc}	3516.7 ^a	3380.6 ^{ab}	3362.1 ^{ab}	202.96	0.0001
Hardness Cycle 2, g	1078.3 ^c	1757.1 ^{bc}	2719.6 ^a	2503.3 ^{ab}	2522.9 ^{ab}	156.43	0.0014
Adhesiveness, mJ	0.183	0.317	0.300	0.322	0.083	0.047	1.0230
Resilience,	0.239	0.258	0.265	0.222	0.214	0.009	0.2834
Cohesiveness	0.381	0.413	0.414	0.330	0.338	0.015	0.2199
Springiness, mm	2.502	2.579	2.734	2.843	2.452	0.328	0.2117
Gumminess, g	511.3 ^c	853.9 ^{bc}	1356 ^a	1009.4 ^{ab}	1068.8 ^{ab}	70.42	0.0001
Chewiness, mJ	13.20 ^b	21.58 ^{ab}	29.13 ^a	24.74 ^a	26.97 ^a	4.886	0.0010

^{a-c} Means in the same row with different superscript letters are significantly different by Tukey's multiple comparison method ($P < 0.05$). C- control diet; A - a diet containing 5% alfalfa meal; AB - a diet containing 5% alfalfa meal and 1% basil; AT - a diet containing 5% alfalfa and 1% thyme; AS - diet containing 5% alfalfa and 1% sage.

CONCLUSION

In conclusion, the results of this study showed that dietary supplementation of basil, thyme, and sage plants together with alfalfa, as feed additives can improve meat quality. They produced a significant increase of total polyphenols content and antioxidant capacity in broiler meat and diminished the cholesterol concentration. Meat colour especially lightness, increases as well as hardness and chewiness and consequently improve the sensorial meat quality.

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