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Chemical composition of dietary alfalfa and its effectiveness on broiler chicken thigh meat quality

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Abstract: This study aimed to evaluate alfalfa meal's chemical and nutritional composition and effectiveness on broiler chicken thigh meat quality. Alfalfa contains significant content of crude protein and fibre, minerals (iron and zinc), polyunsaturated fatty acids (PUFA), and antioxidant compounds, especially total antioxidant capacity (TAC) and total polyphenols content (TPC). To test its effectiveness on chicken tight meat quality, we developed a trial on 60 Cobb 500 broilers, divided into two groups of 30 animals each and fed during growing-finishing phases (11–42 days) a control diet (C) and an experimental diet (A), in which 5% alfalfa meal was added. At the end of the trial, six animals from each group were selected for slaughter and sampling. The analyses on meat samples revealed that alfalfa significantly affected bioactive compounds with antioxidant potential, such as zinc, vitamin E and TPC, compared with the C samples. The utilisation of 5% alfalfa was also very effective on the fatty acids composition of thigh meat samples in the A group by increasing the concentration of eicosapentaenoic acid and significantly decreasing the n-6/n-3 ratio as well as cholesterol content with 10.41% in experimental samples. Therefore, alfalfa can be a potential alternative to synthetic feed additives in producing healthier chicken meat, with increased content of bioactive compounds and essential fatty acids for human health.

Keywords: antioxidants; bioactive compounds; fatty acids; feed additives

In response to consumer concerns about the harmful effect of synthetic feed additives, there is a trend toward substituting them with natural antioxidants, which are both affordable and effective and have a favourable impact on meat quality products and target consumer health. Numerous research has shown in recent years that adding various plants (Vlaicu et al. 2022), extracts (Saracila et al. 2022), or by-products (Untea et al. 2022) to poultry feed, can change the nutritional composition, minerals, vitamins, antioxidants, and other bioactive compounds of the chicken meat. The potential use of natural plants as meat quality modifiers offers an alternative to the use of synthetic substances that are less expensive, safer, more sustainable, and more acceptable to consumers. Bioactive substances such as vitamins, minerals, antioxidants, polyphenols, and essential fatty acids naturally occur in different plant parts and are important constituents of nutraceuticals and functional foods. The biological effects of plants, including antibacterial, anti-inflammatory, immunostimulatory,

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hypocholesterolemic, anticarcinogenic, and antioxidant properties, have caught the interest of researchers all over the world (Bera et al. 2019; Jain et al. 2019).

Alfalfa, also known as *Medicago sativa* L., may be employed as a plant-derived antioxidant ingredient in broiler chicken feed, according to a small number of studies (Bera et al. 2019; Bobko et al. 2021; Kwiecień et al. 2021) with beneficial effects on meat quality. Numerous studies tested the effect of alfalfa as a feed additive in rabbits' meat quality (Baldi et al. 2019), laying hens as a source of carotenoids (Englmaierová et al. 2019) or as gross forage for ruminants (Xu et al. 2023). However, limited information is available on the use of alfalfa as a feed additive in broiler chicken, mostly because of its high dietary fibre content, which limits its usage (He et al. 2021) because it considerably influences production performances.

Thus, the present study determined the chemical and nutritional composition of alfalfa. The addition of 5% alfalfa, as a source of feed additive, in the broiler chicken diet aimed to elucidate its effects on broiler chicken meat quality.

MATERIAL AND METHODS

Experimental design and animals. Alfalfa was purchased from a local producer (Harnes Company, Stefanesti County, Arges) in pelleted form, powdered in a hammer mill with a 1 mm screen, and then included in the compound feed formulation, in the form of powder as the main ingredient and mixed with the other ingredients.

This study was conducted on 60 Cobb 500 broiler chickens purchased from a local hatchery and randomly distributed into 2 homogeneous groups of 30 chickens each (six replicates of five chickens each). According to the sanitary veterinary norms, they were housed in an experimental hall equipped with three-tiered Big Dutchman digestibility cages and controlled microclimate conditions. After the starter phase (10 days), the chickens were weighed individually and assigned to two groups (C and A) with homogenous weights for the grower (11–28 days) and finisher (29–42 days) phases. Corn and soybean meal were used as the main ingredients in the control diet (C), and the experimental diet was supplemented with 5% alfalfa (A) as a meal. The compound feed was given in the mash form to the broilers. The nutritional value of the feed mixtures contained 3 025 kcal·kg^{-1} metabolisable energy, 19.50% crude protein (C and A group) with 3.45% (C group), and 4.43% (A group) crude fiber for the starter phase (10 days). The grower phase (11–28 days) contained 3 100 kcal·kg^{-1} metabolisable energy, 18.50% crude protein (both C and A groups) with 3.31% (C group), and 4.25% (A group) of crude fiber. In the finisher phase (29–42 days) metabolisable energy was 3 150 kcal·kg^{-1} and 17.50% crude protein content (both C and A groups) while crude fiber was 3.15% for C group and 4.08% for A group. After 42 experimental days, 6 broilers per group were slaughtered by cervical dislocation and samples of thigh meat were collected for quality analyses.

Determination of chemical composition. The chemical analysis of alfalfa and chicken thigh meat muscle was carried out following the methods recommended by the Association of Official Analytical Chemists (AOAC 1990). The crude protein was determined by the Kjeldahl method (Kjeltec Auto 1030; Tecator Instruments, Sweden), crude fat was determined by extraction in organic solvents (Soxtec 2055; Foss Tecator, Sweden), crude fibre was determined by the method with intermediary filtration (Fibertec 2010 System; Foss Tecator, Sweden) and ash content was determined by a gravimetric method using a Caloris CL 1206 furnace (Nabertherm, Germany).

Determination of trace mineral composition. The mineral composition of zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn) was determined using atomic absorption spectrometry (FAAS), after microwave digestion, by using SOLAAR M6 Dual Zeeman Comfort (Thermo Electron, UK) equipment, as described by Untea et al. (2012).

Determination of lipophilic and hydrophilic antioxidant compounds. Determination of total phenolic content (TPC) was done spectrophotometrically according to Folin-Ciocalteu's method, and the gallic acid was used for the calibration curve. The results were expressed as mg gallic acid equivalents per gram sample (mg GAE·g^{-1}).

The total antioxidant capacity (TAC) of the extracts was based on the reaction between the sample solution and DPPH reagent prepared in methanol, and the was absorbance recorded at 517 nm using a V-530 Jasco (Japan Servo Co., Japan) spectrophotometer, and the results are expressed as mM Trolox.

Lutein and zeaxanthin content were analysed using high-performance liquid chromatography (HPLC; Perkin Elmer 200 series, Shelton, USA) with a UV detector (445 nm) and a Nucleodur C18 column (Macherey-Nagel, Germany), as described by Varzaru et al. (2015).

Vitamin E determination in meat was performed using a high-performance liquid chromatograph (HPLC Finningan Surveyor Plus; Thermo-Electron

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Corporation, USA) and a PDA-UV detector at a wavelength of 292 nm (Varzaru et al. 2015).

Determination of fatty acids profile and cholesterol concentration. For the fatty acid determination, a gas chromatograph (Clarus 500; Perkin-Elmer, USA), fitted with a flame ionisation detector and capillary separation column with a high polar stationary phase TRACE TR-Fame (Thermo Electron, USA) with dimensions of 60 m × 0.25 mm × 0.25 µm, was used, as described by Turcu et al. (2019). The cholesterol concentration was determined by gas chromatography, with the same Perkin-Elmer Clarus according to AOAC (1990).

Statistical analyses. Obtained data were statistically analysed by one-way analysis of variance (ANOVA) (Stat View for Windows, SAS, version 6.0; Brain Power Inc., USA), calculating mean values and standard error of the mean (SEM) for each sample. The significance of individual mean differences was considered at $P < 0.05$. GraphPad Prism software, version 13.2 (GraphPad Software, USA) was used for graphics representations.

RESULTS AND DISCUSSION

Nutritional and mineral composition, antioxidant compounds and fatty acids profile of alfalfa meal. The results presented in Table 1 revealed that alfalfa is a rich source of crude fibre, having a moderate level of crude protein, and low ether extract content, as similarly reported by others (Laudadio et al. 2014). However, its nutritional composition could differ due to different cultivars or seasonal cultivation and harvesting time. The mineral profile showed that alfalfa is a rich zinc-iron, manganese, and zinc source, with low copper content. These micronutrients are essential for animals' growth and development and are involved in immunologic functions. Ferreira et al. (2015) reported different concentrations of these nutrients in four types of alfalfa cultivars, but their study was based on alfalfa irrigated with water of different electrical conductivities. From the bioactive compounds with antioxidant potential determined in alfalfa, TPC, lutein, and zeaxanthin were dominants, followed by TAC. Generally, alfalfa meal has been used as a source of carotenoids in poultry nutrition to enhance the egg yolk colour or chicken meat skin (Englmaierová et al. 2019). However, recent literature data reported that it can be a considerable source of compounds with antioxidant potential (Chen et al. 2020; Guo et al. 2022). Because alfalfa contains various phytochemicals (flavonoids and carotenoids), which act as antioxidants, they help to neutralise free radicals and support the overall health and well-being of the broilers, indicating its beneficial effects.

Table 1. Nutritional and mineral composition and antioxidant compounds determined in alfalfa

Specification	Alfalfa
Nutritional composition	
Crude protein (%)	17.06
Ether extract (%)	1.13
Crude fibre (%)	26.21
Ash (%)	11.89
Mineral composition	
Copper (mg·kg^{-1})	7.26
Iron (mg·kg^{-1})	2 318.18
Manganese (mg·kg^{-1})	57.12
Zinc (mg·kg^{-1})	31.24
Antioxidant compounds	
TPC (mg GAE·g^{-1})	31.24
TAC (mM Trolox)	17.78
Lutein and zeaxanthin (mg·kg^{-1})	28.53
Vitamin E (mg·kg^{-1})	53.17

TPC – total polyphenols content; TAC – total antioxidant capacity; GAE – gallic acid equivalents

The fatty acids profile presented in Table 2 revealed that more than half of total fatty acids determined in alfalfa were represented by polyunsaturated fatty acids (PUFA), especially n-3 ALA, and lower content of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). A previous study reported that alfalfa contained 27 g·(100 g)$^{-1}$ SFA, 8.3 g·(100 g)$^{-1}$ MUFA, and 61.9 g·(100 g)$^{-1}$ PUFA (Leiber et al. 2008). As in our case, the authors reported that the n-3 ALA accounted for more than half of the total PUFA [39.2 g·(100 g)$^{-1}$]. Although little attention has been given to the fatty acid profile of alfalfa, we observed that alfalfa contains a mix of n-3 and n-6 fatty acids. The content of n-3 ALA in alfalfa [41.36 g·(100 g)$^{-1}$] is almost similar with the content determined in *Camelina sativa* [43.25 g·(100 g)$^{-1}$] and *Linum usitatissimum* [42.93 g·(100 g)$^{-1}$] (Colonna et al. 2021; Vlaicu et al. 2021), which are well recognised sources of essential fatty acids. In this light, alfalfa can be considered a good source of essential fatty acids for broiler chickens, which are beneficial for their overall health and meat enhancement.

Effect of alfalfa on the primary and mineral composition of thigh meat samples. Alfalfa meal had no significant impact on the nutritional composition

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Table 2. Fatty acid profile of the alfalfa meal

Fatty acids [g·(100 g)$^{-1}$ FA]	Alfalfa
Caproic C6:0	0.34
Caprylic C8:0	0.53
Capric C10:0	0.40
Lauric C12:0	0.83
Myristic C14:0	1.46
Pentadecanoic C15:0	0.44
Palmitic C16:0	22.08
Heptadecanoic C17:0	0.32
Stearic C18:0	3.18
Tricosanoic C23:0	0.15
∑SFA	29.73
Myristioleic C14:1	0.03
Pentadecenoic C15:1	2.79
Palmitoleic C16:1	2.30
Heptadecenoic C17:1	0.63
Oleic cis C18:1	4.05
Nervonic C24:1n9	0.07
∑MUFA	9.87
Linoleic cis C18:2n6	16.88
Linolenic γ C18:3n6	0.28
Eicosadienoic C20:2n6	0.28
Arachidonic C20:4n6	0.30
∑n-6 PUFA	17.75
Linolenic α C18:3n3	41.36
Octadecatetraenoic C18:4n3	0.61
∑n-3 PUFA	41.97
PUFA	59.72
Others	0.68
n-6/n-3	0.42

FA – fatty acids; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; n-3 – total omega 3 fatty acids; n-6 – total omega 6 fatty acids

of thigh meat compared with control samples (Table 3). In terms of mineral composition, although alfalfa was observed to be a rich source of iron, this was not reflected in the meat samples. At the same time, zinc concentration was significantly higher ($P < 0.05$) in the A samples compared with the C samples. This might be explained by the fact that chickens have high iron demands for optimal growth during growing. It can be assumed that the high iron content in alfalfa is due to soil rich in iron. The mineral bioavailability in animal organisms is a complex process, influenced by many factors and with a small yield (Bao and Choct 2009).

Table 3. Chemical composition of meat samples

Specification	C	A	SEM	*P*-value
Nutritional composition				
Dry matter (%)	28.23	27.79	0.786	0.7951
Crude protein (%)	18.33	17.86	0.498	0.6555
Ether extract (%)	8.36	8.34	0.331	0.9682
Ash (%)	1.12	1.15	0.038	0.7007
Mineral composition				
Copper (mg·kg^{-1})	1.22	1.29	0.099	0.6706
Iron (mg·kg^{-1})	38.14	39.17	0.770	0.0579
Manganese (mg·kg^{-1})	0.10	0.12	0.017	0.5135
Zinc (mg·kg^{-1})	50.63^{b}	54.79^{a}	0.873	0.0061

$^{a, b}$ means in the same row with different superscript letters are significantly different at $P < 0.05$; C – control diet; A – experimental diet; SEM – standard error of the mean; *P* – significance

The same researchers proved that iron and zinc interact at the intestinal mucosa level and impair the absorption of each other (Bao and Choct 2009).

However, recently it was reported (Lu et al. 2022) that it is not clear whether the difference in iron bioavailability is due to the differences in iron absorption, metabolic utilisation, or both aspects. On the other hand, zinc from organic sources has higher bioavailability than zinc from inorganic sources, and it is proven that it has major implications in enhancing antioxidant capacity, modulating immunity, and improving health indices in broiler chickens (Ogbuewu and Mbajiorgu 2023). It was suggested (Kondaiah et al. 2019) that competition at a particular protein transport pathway during intestinal absorption causes this type of iron-zinc interactions. Iron and zinc synergistically contribute to the growth and development of broilers, fostering improved nutrient absorption and utilisation. Zinc, in particular, plays a key role as a modulator of intestinal absorption and tissue distribution of iron. This interaction between iron and zinc underscores their combined impact on optimising broiler health and maximising their growth potential through enhanced nutrient uptake and utilisation.

Effect of alfalfa on fatty acids profile and cholesterol content determined in thigh meat samples. In the thigh meat, the MUFA represented the most abundant group of fatty acids, followed by SFA and PUFA (Table 4). The SFA were significantly higher ($P < 0.05$) in the C samples compared with the A samples. There were no significant differences among the two groups for the sums of the MUFA, where the oleic acid was the

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Table 4. Fatty acid profile and cholesterol content of the thigh meat samples

Fatty acids [g·(100 g)$^{-1}$]	C	A	SEM	*P*
Butyric C4:0	0.55	0.49	0.022	0.2319
Caproic C6:0	0.46	0.43	0.019	0.4578
Caprylic C8:0	0.71	0.67	0.065	0.7459
Capric C10:0	0.42	0.46	0.042	0.6394
Lauric C12:0	0.02	0.03	0.002	0.4178
Miristic C14:0	1.35	1.33	0.064	0.8262
Pentadecanoic C15:0	0.47^{b}	0.52^{a}	0.012	0.0139
Palmitic C16:0	25.75^{a}	24.78^{b}	0.163	< 0.0001
Heptadecanoic C17:0	0.08^{b}	0.11^{a}	0.007	0.0281
Stearic C18:0	10.07^{b}	10.38^{a}	0.073	0.0227
Lignoceric C24:0	0.80	0.84	0.020	0.2753
∑SFA	41.11^{a}	40.05^{b}	0.257	0.0291
Myristioleic C14:1	0.10	0.11	0.003	0.1828
Pentadecenoic C15:1	1.38^{b}	1.78^{a}	0.067	< 0.0001
Palmitoleic C16:1	3.22^{a}	2.53^{b}	0.115	< 0.0001
Heptadecenoic C17:1	0.32^{a}	0.28^{b}	0.011	0.0498
Oleic cis C18:1n9	39.44	39.53	0.151	0.7686
∑MUFA	44.36	44.27	0.162	0.7999
Linoleic cis C18:2n6	7.31^{b}	7.97^{a}	0.141	0.0090
Linolenic γ C18:3n6	0.06	0.05	0.006	0.6723
Conjugated LA C18:2	0.68	0.76	0.043	0.3439
Eicosadienoic C20:2n6	2.51	2.50	0.087	0.9700
Arachidonic C20:4n6	0.13	0.16	0.012	0.2852
Docosadienoic C22:2n6	0.57	0.49	0.023	0.0958
Docosatrienoic C22:3n6	0.69	0.63	0.019	0.0990
∑n-6 PUFA	11.93^{b}	12.34^{a}	0.154	0.0426
Linolenic α C18:3n3	0.11	0.17	0.008	0.6524
Octadecatetraenoic C20:4n3	1.48	1.58	0.041	0.2611
Eicosapentaenoic C20:5n3	0.72^{b}	0.84^{a}	0.023	0.0007
∑n-3 PUFA	2.32^{b}	2.59^{a}	0.041	0.0044
∑PUFA	14.25	14.93	0.248	0.0534
n-6/n-3	5.14^{a}	4.76^{b}	0.067	0.0390

$^{a, b}$ means in the same row with different superscript letters are significantly different at $P < 0.05$; C – control diet; A – experimental diet; SEM – standard error of the mean; *P* – significance; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; n-6 – total omega 6 fatty acids; n-3 – total omega 3 fatty acids

most abundant, accounting for almost 89% of the total sum. Alfalfa contributed to significantly altering some fatty acids in the PUFA group. From the n-6 PUFA, linoleic fatty acid (LNA – C18:2n6) was significantly higher ($P < 0.05$) in the A meat samples than in the C samples, leading to a significantly higher ($P < 0.05$) content of n-6 PUFA. From the n-3 PUFA group, the eicosapentaenoic essential fatty acid (EPA – C20:5n3) was 14.28% higher in the A samples than in the C samples. These alterations among the groups of fatty acids led to a significantly lower ($P < 0.05$) n-6/n-3 ratio in the A samples compared with those from the C group. Concomitantly, it led to significantly lower cholesterol content in the A meat samples compared to the C samples. It is already known that incorporating essential PUFA in the human diet plays a natural preventive role in cardiovascular disease and other health problems. In this context, dietary alfalfa significantly improved the thigh meat fatty acids profile because of increased health-promoting fatty acids such as LNA and EPA.

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The modulation of the fatty acids composition in chicken meat was successfully done previously when dietary fat sources (Untea et al. 2022), plants (Vlaicu et al. 2022) or vegetable sources (Gheorghe et al. 2019) were used in their diets. Although little is known about alfalfa's potential to enhance the fatty acids profile in chicken meat, more attention was given to alfalfa as a dietary source for rabbits (Leiber et al. 2008) and lambs (Baldi et al. 2019), resulting in beneficial effects. In this study, the increased concentration of EPA in the A meat samples is attributed to the conversion through elongation of its metabolic precursor (ALA), which was determined in significant concentration in the alfalfa. Recently, it was reported that 15 g·kg^{-1} of alfalfa was efficient at increasing the quality of n-3 PUFA in male broilers thigh meat, compared with the control diet, and lowered the ratio of n-6/n-3. However, their value was almost double (9.10) than our results (Kwiecień et al. 2021). Since the n-6/n-3 ratio is a quality indicator of meat, it should be around four to be recommended as beneficial to human consumers. Although the previously mentioned study reported improved meat quality regarding n-3 PUFA, their n-6/n-3 ratio indicated that the amount of n-6 PUFA was higher compared with the results presented in this paper. These different effects between the studies, might be attributed to the chemical composition of alfalfa and other factors that may have impact on this parameter.

When considering cholesterol content, a lower amount in thigh muscle becomes advantageous for consumers as it contributes to reduce the risk of cardiovascular disease, signifying a superior quality and a healthier product (Figure 1). While the specific impact of alfalfa on cholesterol content in meat remains relatively unexplored, Carrasco et al. (2018) observed comparable results, crediting the presence of saponins in alfalfa for the cholesterol-lowering effects. However, we consider that since alfalfa contains high content of ALA, which was converted to EPA, it might be associated with a decrease in cholesterol levels in chicken meat. Moreover, by providing chickens with diets that have a more balanced n-6 to n-3 ratio, the cholesterol-lowering effect of n-3 fatty acids in chicken meat can be enhanced. Beneficial impacts of improved cholesterol levels and a decreased occurrence of coronary heart diseases as influenced by the various fatty acids, has been recently summarised (Alagawany et al. 2022). These promising findings highlight the potential role of alfalfa as a beneficial dietary factor for enhancing meat quality and promoting consumer well-being.

Effect of alfalfa on total polyphenols content, antioxidant capacity, lutein and zeaxanthin, and vitamin E, determined in thigh meat samples. From the bioactive compounds with antioxidant potential (Figure 2), TPC and vitamin E significantly increased in the A meat samples, with no effect on TAC, lutein, or zeaxanthin. Alfalfa is known to be a rich source of polyphenolic compounds, including flavonoids and phenolic acids. These compounds are well-known antioxidants and can contribute to the overall antioxidant capacity of foods. Broilers consuming alfalfa resulted in increased levels of polyphenols transfer in their thigh meat tissues. Both TPC and TAC are well-known for their antioxidant properties, to neutralise free radicals and reduce oxidative stress in the body. However, when the concentration of TPC is too high, it may lead to a decrease in the overall TAC, because excessive polyphenols can compete with other antioxidants for binding sites on free radicals, reducing the overall effectiveness of the antioxidant capacity (Halliwell 2007). Further, the presence of vitamin E in alfalfa also resulted in deposition of this antioxidant in thigh meat samples. Similarly, other authors reported that 4% of alfalfa used in Ross 308 and Cobb 500 broiler chickens effectively increased the biologically active substances in chicken meat samples (Tkáčová et al. 2015). A study with laying hens reported that alfalfa significantly increased the concentration of vitamin A, lutein, and zeaxanthin

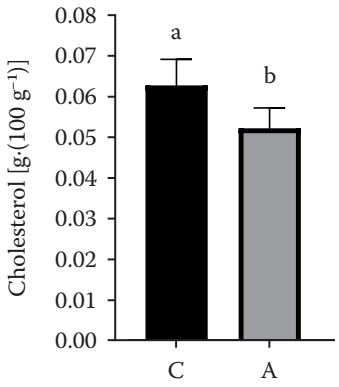

Figure 1. Effect of alfalfa on cholesterol content in thigh meat

a, b – significant difference among the groups at $P < 0.05$; C – control diet; A – experimental diet with 5% alfalfa

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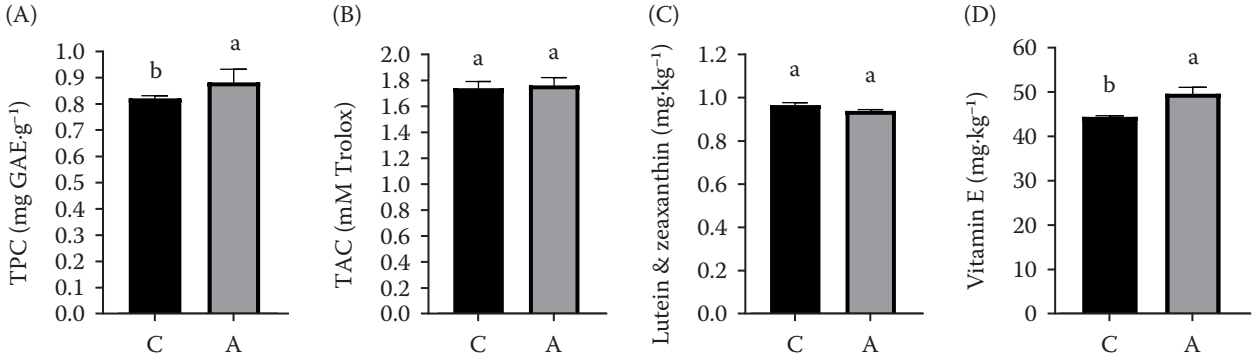

Figure 2. Effect of alfalfa on (A) total polyphenols (TPC), (B) antioxidant capacity (TAC), (C) lutein and zeaxanthin, and (D) vitamin E content in thigh meat

a, b – significant difference among the groups at $P < 0.05$; C – control diet; A – experimental diet with 5% alfalfa; GAE – gallic acid equivalents

in the yolks (Englmaierová et al. 2019). Based on these findings, we can point out that during their transfer from the feed to the food matrix, they may be subjected to isomerisation, resulting in different transfer rates of these compounds. Lutein and zeaxanthin, as well as vitamin E, are all fat-soluble antioxidants. When consumed together in high amounts, they may compete for absorption and utilisation in the body, potentially resulting in antagonistic effects and reduced availability of one or more of these compounds. The same effect was recently reported by Saracila et al. (2022), however, in our case vitamin E was higher and lower concentration of lutein and zeaxanthin were determined, which confirms a possible antagonist effect between these bioactive compounds.

CONCLUSION

In conclusion, including 5% alfalfa in broiler chicken diets significantly improves meat quality by enhancing antioxidant potential and positively influencing long-chain PUFA and cholesterol content. Breeders and farmers should consider incorporating up to 5% alfalfa to enhance the nutritional profile and quality of the meat. However, chemical analysis and consideration of regional variations are necessary to determine the viability of alfalfa inclusion in broiler diets.

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