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The effect of dietary supplementation with leaves of industrial hemp (*Canabis sativa* L.) on the carcass and meat quality traits of broiler breast meat

Utjecaj dodatka lišća industrijske konoplje (*Canabis sativa* L.) u krmnu smjesu na karakteristike trupa i kakvoću mesa tovnih pilića

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ABSTRACT

The study aimed to determine the effect of dietary supplementation with leaves of industrial hemp (*Cannabis sativa* L.) on live weight, carcass (carcass weight, dressing percentage, individual cuts and giblets) and meat quality (pH, color, EZ-DripLoss, thawing loss, cooking loss, and shear force) traits of broiler chicken meat. The study was conducted on 100 male Ross 308 chickens, which were divided into 4 experimental groups. The control group (K-0) was fed without hemp leaves, while groups P-1, P-2 and P-3 were fed with the addition of 1%, 2%, and 3% industrial hemp leaves, respectively. The animals were slaughtered at 42 days of age. Group P-2 had the highest wing proportion (10.30%), which was significantly different from the group P-1 (9.43%; *P* = 0.004). Abdominal fat was significantly lower in groups P-2 (0.66%) and P-3 (0.59%) compared to group P-1 (1.06%; *P* = 0.004). The breast meat of group P-2 was the lightest (L* = 48.28) and differed significantly from that of group P-1 (L* = 46.24; *P* = 0.044). Group P-1 had the highest shear force value (17.22 N), which was significantly different from groups P-2 (*P* = 0.0047) and P-3 (*P* = 0.0506). Hemp leaves may improve the quality of broiler meat, but more research is needed to understand their full effect on other traits and health.

Keywords: feed, Ross 308, carcass traits, physical traits

SAŽETAK

Cilj ovog istraživanja bio je utvrditi učinak dodatka lišća industrijske konoplje (*Cannabis sativa* L.) u krmnu smjesu na živu masu, karakteristike trupa (klaonička masa, randman, dijelovi trupa i unutarnji organi) i kakvoću mesa (pH vrijednost, boja, EZ-DripLoss, kalo odmrzavanja, kalo kuhanja i sila presijecanja) mesa tovnih pilića. Istraživanje je provedeno na 100 muških jedinki hibridne linije Ross 308, podijeljenih u 4 eksperimentalne skupine. Kontrolna skupina (K-0) hranjena je bez dodatka listova konoplje, dok su skupine P-1, P-2 i P-3 hranjene s dodatkom od 1%, 2% i 3% listova industrijske konoplje. Životinje su zaklane u dobi od 42 dana. Skupina P-2 imala je najveći udio krila (10,30%), što je bilo statistički značajno u usporedbi sa skupinom P-1 (9,43%; *P* = 0,004). Udio abdominalne masnoće bilo je značajno niži u skupinama P-2 (0,66%) i P-3 (0,59%) u usporedbi sa skupinom P-1 (1,06%; *P* = 0,004). Meso prsa iz skupine P-2 bilo je najsvjetlije (L* = 48,28) i značajno se razlikovalo od mesa skupine P-1 (L* = 46,24; *P* = 0,044). Skupina P-1 imala je najvišu vrijednost sile presijecanja (17,22 N), što je bilo značajno u usporedbi sa skupinama P-2 (*P* = 0,0047) i P-3 (*P* = 0,0506). Dodatak listova konoplje u krmnu smjesu može poboljšati kvalitetu mesa peradi, ali potrebno je više istraživanja kako bi se u potpunosti razumio njihov utjecaj na ostala svojstva i cjelokupno zdravlje peradi.

Ključne riječi: hrana, Ross 308, klaonički pokazatelji, fizikalna svojstva

INTRODUCTION

In recent years, the agricultural industry has experienced a growing interest in sustainable and innovative feeding strategies that improve livestock health and product quality while adhering to environmental and ethical standards. Among these novel feed additives currently being researched, *Cannabis sativa* L., predominantly in its non-psychoactive form known as industrial hemp, has attracted considerable attention. As one of the oldest cultivated plants, hemp thrives throughout the Northern Hemisphere and serves a variety of industrial purposes as well as a staple food for humans and animals (Muedi et al., 2024). It has attracted attention due to its rapid growth, high biomass production, rich nutritional profile, and potential health benefits (Fallahi et al., 2022). In fact, hemp contains, in addition to its processing by-products such as seeds, oil, oilseed cake, hulls, flour and leaves, a wide variety of components rich in active compounds, including mainly phytocannabinoids, terpenes, terpenoids, and flavonoids. In addition, hemp contains a considerable concentration of essential fatty acids, proteins, carbohydrates, chlorophylls, vitamins, minerals, and other substances (Della Rocca and Di Salvo, 2020). The nutritional value, health benefits, and industrial applications of hemp are described in detail by Lanzoni et al. (2024) and Muedi et al. (2024). Due to its beneficial effects the integration of *Cannabis sativa* L. into the diet of livestock, especially broilers, has recently received increased attention.

As observed in the study by Khan et al. (2010), broilers fed a hemp seed-based diet at different inclusion levels (5%, 10%, and 20%) showed better body weight gain, feed intake, and feed conversion ratio than the broilers fed the control diet. At the 20% inclusion level, the broilers gained more weight (2087.2 ± 10.25 g), due to a better feed conversion ratio (1.95 ± 0.032) while they consumed less feed than the control diet (4070.2 ± 20.2 vs. 5014.4 ± 6.3 g). However, at the 5% inclusion level, broilers consumed more feed (4506.9 ± 91.9 g) than all other diets containing hemp seed. Parr et al. (2020) also reported that the addition of 20% hemp hearts to the diet was more effective than 10%, 30%, and 40% in modulating the growth performance of broilers, i.e. feed conversion rate and net weight gain. Jing et al. (2017) showed that the addition of 3% and 6% hemp seed oil to a control diet (based on corn oil) did not change the performance (body weight, feed intake, and feed conversion ratio) of broilers, but increased the content of n-3 polyunsaturated fatty acids (PUFAs) in their meat (71.4 ± 14.8 g/100 g and 148.9 ± 14.8 g/100 g, respectively). Šťastník et al. (2016) investigated the use of 2.5% hempseed expeller and 1% pellets from the tops of technical hemp plants in broiler diets and found no difference in the final weight and carcass yield compared to the control group fed with maize diet. Eriksson and Wall (2012) found that the inclusion of 20% hempseed cake in broiler diets did not result in significant differences in production performance parameters such as feed intake, feed conversion ratio, final weight, or mortality rate compared to control diets containing soybean cake, rapeseed cake, and peas.

However, Šťastník et al. (2015) reported the opposite results when adding 5% and 15% hempseed cake to feed mixtures, which led to a decrease in final weight compared to the control feed (soybean cake). As suggested by Lanzoni et al. (2024) these effects are probably due to antinutritional factors, especially non-starch polysaccharides, whose fibrous content increases with the removal of the lipid fraction.

In addition, reports from the literature agree that the inclusion of hemp meal in poultry feed can effectively improve the nutritional value of meat and obtained food products (higher total n-3 PUFAs proportion, higher PUFA to saturated fatty acids (SFA) ratio, and a more balanced n-6/n-3 PUFAs ratio), color, and sensory properties compared to the poultry food products obtained from animals fed a normal diet (Farinon et al., 2020; Yalcin et al., 2018). According to Palmquist (2009) and Banskota et al. (2022), hemp seeds and oil have an excellent PUFA profile, which has been associated with an improvement in the nutritional quality and sensory properties of broiler meat. Šťastník et al. (2019) found that the addition of hempseed expellers at levels of 5% and 15% to broiler feed resulted in meat color (by increasing red and yellow parameters) and odor values of breast meat, thus improving meat quality. Kaić et al. (2024) found that supplementation with industrial hemp leaves at levels of 1%, 2%, and 3% did not affect the pH value, thawing loss or cooking loss of broiler breast meat, except the yellow color parameter, which increased in the group supplemented with 1% hemp leaves.

In addition, some studies have emphasized the antioxidant potential of hemp in reducing oxidative stress in poultry, suggesting that the inclusion of hemp in the poultry diets may improve the antioxidant status of the animal, thereby improving meat quality and extending shelf life (Farinon et al., 2020; Semwogerere et al., 2020).

While considerable research has focused on the use of hemp seed, cake and oil, the potential of hemp leaf as a feed additive has not been sufficiently explored, particularly with regard to its effects on carcass and meat quality traits in poultry. Muedi et al. (2024), Fallahi et al. (2022), and Lanzoni et al. (2024) indicated that further research is needed to evaluate the effect of hemp leaf meal on the production performance and meat quality of livestock species. The aim of the study was therefore to determine the effect of dietary supplementation of industrial hemp leaf (*Cannabis sativa* L.) on carcass and meat quality traits of broiler meat.

MATERIAL AND METHODS

Animals, study design, and diets

The study was conducted on 100 male Ross 308 broilers, which were divided into four experimental groups with 25 chicks per group and reared over a period of 42 days. The broilers were housed on the floor in pens filled with dry wood shavings litter (approx. 10 cm thick), with a density of 33 kg/m^2 according to technological standards. The housing conditions were standardized for all groups. The ventilation of the facility was based on natural airflow due to gravity, while the broilers were kept warm using 250 W infrared lamps. The lighting system was set to a cycle of 23-h of light and 1-h of darkness. The initial temperature in the broiler area was set at 32 °C, and gradually decreased to 24 °C following standard procedures.

The broilers were fed *ad libitum* with a feed mixture (Table 1) as follows, from day 1 to day 21, they received a starter feed mixture (Table 2), and then a finisher feed mixture (Table 2) from the day 22 to the day 42.

Table 1. The composition of the experimental diets

Ingredient composition (g/kg)	Feed mixture[1]			
	K-0	P-1	P-2	P-3
Maize	55.90	56.00	55.40	54.40
Soybean meal	32.40	31.10	30.70	30.70
Soybean cake	3.30	3.50	3.50	3.50
Sunflower meal	2.10	2.10	2.10	2.10
Hemp leaves	0.00	1.00	2.00	3.00
Sunflower oil	2.90	2.90	2.90	2.90
$CaCO_3$	1.30	1.30	1.30	1.30
Dicalcium phosphate	1.30	1.30	1.30	1.30
Sodium chloride	0.25	0.25	0.25	0.25
Vitamin mineral premix[2]	0.50	0.50	0.50	0.50
DL Methionine	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00

[1] K-0: Feed mixture without hemp addition; P-1: Feed mixture with 1% hemp addition; P-2: Feed mixture with 2% hemp addition; P-3: Feed mixture with 3% hemp addition;

[2] One kg of premix contains: vit. A 2.700.000 IJ; vit. D3 400.000 IJ; vit. E 6.000 mg; vit. K3 500 mg; vit. B1 200 mg; vit B2 1.000 mg; vit. B6 400 mg; vit. B12 3.000 mcg; vit. C 3.000 mg; niacin 8.000 mg; pantothenic acid 2.400 mg; folic acid 100 mg; biotin 20 mg; choline chloride 100.000 mg; iron 7.200 mg; iodine 150 mg; copper 800 mg; manganese 15.000 mg; zinc 10.000 mg; selenium 30 mg.

The main feed ratio were formulated to meet the nutritional needs of broilers based on the Aviagen nutritional requirements (Aviagen Inc. Broiler Nutrition Specifications, 2022). The control group (K-0) received a feed mixture without hemp leaves, while the three experimental groups had their feed supplemented with industrial hemp leaves at concentrations of 1% (P-1 group), 2% (P-2 group), and 3% (P-3 group). Water was provided *ad libitum* via nipple drinkers throughout the experiment. Industrial hemp from GEA-COM Ltd. (Budačka Rijeka, Croatia) was harvested when the plants were four months old, indicating they were in the reproductive phase.

Table 2. The chemical composition of starter and finisher feed mixture

Nutrient content	Starter				Finisher			
	K-0	P-1	P-2	P-3	K-0	P-1	P-2	P-3
Crude protein, %	20.37	20.47	20.47	20.68	17.72	17.72	17.44	17.84
Crude fibre, %	4.49	4.13	4.38	4.79	4.29	4.15	4.24	4.57
Ash, %	5.45	5.66	5.94	6.02	3.84	3.76	4.02	4.04
Total fat, %	5.26	6.05	6.13	6.09	4.31	5.06	5.23	5.23
Water, %	11.15	10.19	10.34	10.41	12.91	12.70	12.66	12.32
Starch, %	42.33	38.42	41.18	42.90	46.33	45.76	45.76	45.76
Sugar content, %	3.96	4.14	3.94	4.15	2.99	2.97	2.97	2.94
Ca, %	0.98	0.93	1.02	1.00	0.54	0.54	0.64	0.64
P, %	0.73	0.71	0.74	0.71	0.45	0.45	0.47	0.46
Na, %	0.19	0.16	0.17	0.18	0.15	0.16	0.15	0.15
Mg, %	0.20	0.18	0.20	0.19	0.16	0.17	0.17	0.17
K, %	0.10	1.11	1.06	1.01	0.68	0.68	0.69	0.72
Cu, mg/kg	13.00	14.00	13.00	13.00	26.00	28.00	25.00	23.00
Mn, mg/kg	110.0	104.0	110.0	106.0	130.0	124.0	134.0	128.0
Zn, mg/kg	144.0	96.0	96.0	92.0	146.0	148.0	150.0	160.0
Fe, mg/kg	362.0	344.0	372.0	322.0	244.0	252.0	248.0	292.0
ME *, MJ/kg	12.54	12.20	12.66	12.99	12.35	12.50	12.52	12.57

K-0: Feed mixture without hemp addition; P-1: Feed mixture with 1% hemp addition; P-2: Feed mixture with 2% hemp addition; P-3: Feed mixture with 3% hemp addition; *ME: metabolizable energy

The leaves were manually separated, sun-dried, and ground into powder. The main nutrient contents of the industrial hemp leaves are shown in Table 3.

Slaughtering procedure, sample collection, and meat quality parameters analysis

At the end of the experiment (at 42 days of age), ten broiler chickens were randomly selected from each group, weighed, and slaughtered by decapitation. After slaughter, the carcasses were eviscerated and weighed, and the carcass yield was calculated. The cleaned carcasses without internal organs were then divided into individual cuts (wing, drumstick, thigh, breast, and back) after chilling for 24 hours at +4 °C. The heart, liver, and gizzard (giblets) were also removed during evisceration. The individual carcass cuts and giblets were weighed, and their percentages were calculated relative to the eviscerated carcass weight (for carcass cuts) or the live weight (for giblets). To determine meat quality traits, ten samples of the *M. pectoralis superficialis* were taken from each carcass and labelled. The values of pH, meat color, and EZ-DripLoss (DL) were measured on the fresh left side of the *pectoralis superficialis* muscle samples, while the right side of the same muscle samples was stored at -20 °C until analysis. Prior to analyzing thawing loss (TL), cooking loss (CL), and Warner-Bratzler shear force (WBSF), the samples were placed in a refrigerator where they were thawed under conventional conditions (+4 °C for 24 hours).

Table 3. The main nutrient contents in original dry matter of industrial hemp (*Canabis sativa* L.) leaves

Nutrient content	Industrial hemp leaves (*Canabis sativa* L.)
Crude protein, %	18.92
Crude fibre, %	15.75
Ash, %	14.34
Total fat, %	9.69
Water, %	5.26
Ca, %	4.12
P, %	0.43
Na, %	0.017
Mg, %	0.58
K, %	1.61
Cu, mg/kg	15.10
Mn, mg/kg	35.53
Zn, mg/kg	47.96
Fe, mg/kg	186.53

pH value

The pH value of the samples was measured 1 and 24 hours *post-mortem* using a portable pH meter (Seven2Go, Mettler Toledo, Switzerland) equipped with a penetration glass probe. Prior to the measurements, the device was calibrated with buffer solutions with pH values of 4.00 and 7.00 (Mettler Toledo, Switzerland). To determine the pH value the probe was inserted into the center of the *pectoralis superficialis* muscle 0.5 to 1 cm below the muscle surface and the pH value was read when the probe was stable.

Color

Muscle color parameters were measured on the skinned surface of the *pectoralis superficialis* muscle, which was free of any carcass defects, after a 1-hour blooming period using a Minolta CR-300 chroma meter (Konica Minolta Sensing Inc., Osaka, Japan). Before the measurements, the chroma meter was calibrated with a standard white ceramic tile (Y = 93.6. x = 0.3129. and y = 0.3193). The color was expressed according to the CIE Lab system, measuring lightness (L*), redness (a*), and yellowness (b*). Three measurements were taken for each sample and the mean value was used for further analysis.

EZ-DripLoss

The muscle samples for the DL analyses were taken 24 hours *post-mortem* from the cranial edge of the *pectoralis superficialis* muscle of each breast. For DL determination, two 2.5 cm thick slices were cut from which cylindrical muscle cores were taken in duplicate (n = 80). The muscle cores with a diameter of 2.5 cm were removed with a special circular knife in vertical fiber orientation (Rasmussen and Anderson, 1996). Before storage, the muscle cores were weighed ($\bar{x}$ = 8.9 g) and once again after each interval (24, 48, and 72 hours). Before each weighing of samples, the surface of the muscle cores of all samples was carefully dabbed with paper (Correa et al., 2007). During the entire experiment, all samples were stored in the same refrigerator at an average temperature of +3.5 °C and an average humidity of 85%. The DL assessment was based on the change in weight of the samples. The mean value of the muscle cores taken in duplicate was used for each DL assessment.

Thawing loss

Before analyzing the TL, the samples were placed in a refrigeration unit where they were thawed under conventional conditions (at +4 °C for 24 hours). After thawing, the samples were removed from the bags, dried with paper, and reweighed. The TL was expressed as the percentage weight loss of the thawed sample relative to the initial sample weight.

Cooking loss

For CL determination, samples were weighed, placed in vacuum-packaged bags, and cooked in a preheated water bath (JB Nova, Grant Instruments Ltd., Shepreth, Cambridgeshire, UK) at 85 °C until they reached an internal temperature of 75 °C, which was monitored by thermocouples connected to a data logger. Once cooked, the samples were cooled under running tape water, refrigerated at 4 °C until equilibrated, dried with paper, and weighed again. The CL was calculated as the difference in weight between cooked and raw samples and expressed as a percentage of the initial weight before cooking.

Warner-Bratzler shear force (WBSF)

The WBSF determination was performed using an Instron Universal Testing Machine (Model 3345, Instron, Canton, MA, USA) calibrated to full scale with a 500 Newton load cell, a crosshead speed of 250 mm/minute, and a sampling rate of 10 points/second. Ten square cores (1 x 1 x 1.5 cm) from each pre-cooked sample (for CL determination) were cut parallel to the muscle fibers and sheared once perpendicular to the long axis of the fibers using a Warner Bratzler V-shaped blade. The WBSF was measured as the mean of the ten peak force measurements on the muscle cores of each sample and expressed in Newtons.

Statistical analysis

The effect of dietary supplementation with industrial hemp leaves on carcass and meat quality traits of broiler meat was tested using the general linear model procedure (GLM) of SAS 9.4 (Statistical Analysis System, 2013) with different treatments as fixed effects. Post-hoc comparison of least square means between the different levels of dietary supplementation was performed with a Bonferroni multiple test correction. Differences were considered statistically significant if $P < 0.05$.

RESULTS

The carcass characteristics of broilers fed different proportions of hemp leaves in the diet are shown in Table 4. The mean LBW of the broilers ranged from 2952.20 g to 3125.30 g. There was no difference in the LBW of chicks selected for slaughter ($P = 0.467$) or in their CW ($P = 0.469$). These non-significant differences were reflected in DP values, which also did not differ between the experimental groups of chicks ($P = 0.787$). The analysis of the individual carcass cuts showed that the proportion of wings was highest in the carcasses from the P-2 (10.30%), showing a significant difference from the P-1 group (9.43%; $P = 0.004$). Non-significant differences were found between groups in other carcass cuts, i.e. drumstick, thigh, breast, and back (Table 4). In the present study, the carcasses of the P-2 group (0.66%) and the P-3 group (0.59%) had significantly lower proportions of abdominal fat than those of the P-1 group (1.06%; $P = 0.004$). Conversely, there was no significant difference in the proportion of abdominal fat between the K-0 (0.73%) and P-1 (1.06%) groups. Likewise, no significant differences between the experimental groups were found in the giblets, i.e. heart, liver, and gizzard ($P > 0.05$; Table 4).

The analysis of the pH values and color parameters of broiler breast meat fed with different proportions of hemp leaves in the feed is shown in Table 5. No significant differences were found between the experimental groups in the pH values measured one hour and 24 hours *post-mortem* ($P > 0.05$). The analysis of the color parameters of the breast meat, i.e. lightness (L*), redness (a*), and yellowness (b*), only showed significant differences in the parameter L* of the breast muscles. The breast meat of the chicks from the P-2 group was the lightest (L* = 48.28) and differed significantly from the P-1 group (L* = 46.24; $P = 0.044$). No significant differences in L* values were found between the other experimental groups ($P > 0.05$).

The analysis of WHC, including DL, CL, and TL, and tenderness of broiler breast meat supplemented with different proportions of hemp leaves in the diet is shown in Table 6. No significant differences were observed in the WHC attributes of broiler meat supplemented with different proportions of hemp leaves ($P > 0.05$; Table 6). Although the DL values did not show significant differences ($P > 0.05$), the results indicate that the breast meat of the P-1 group had slightly lower DL values than other experimental groups at 24 hours, 48 hours, and 72 hours *post-mortem*. In addition, it should be noted that the DL values in the present study showed a positive linear trend, increasing over time within each experimental group across all investigated measurement intervals (Table 6).

Table 4. The effect of dietary supplementation of industrial hemp leaves on the carcass characteristics of broiler breast meat (LSM values)

Trait (LSM)	K-0	P-1	P-2	P-3	*P*-value
			Treatment		
LBW, g	3035.40	3125.30	3077.90	2952.20	0.4677
CW, g	2338.10	2425.10	2366.70	2270.70	0.4692
DP, %	77.02	77.58	76.80	76.84	0.7878
			Proportion in chilled carcass (%)		
Wing, %	9.64^{ab}	9.43^{a}	10.30^{b}	10.09^{b}	0.0028
Drumstick, %	12.08	12.19	12.73	25.59	0.4014
Thigh, %	15.10	15.15	14.87	15.12	0.8881
Breast, %	32.71	32.16	30.91	31.20	0.9198
Back, %	22.07	22.85	22.33	22.46	0.7088
Abdominal fat, %	0.73^{ab}	1.06^{a}	0.66^{b}	0.59^{b}	0.0041
			Proportion in live body weight (%)		
Hearth, %	0.46	0.47	0.47	0.47	0.8093
Liver, %	2.01	1.92	2.05	1.86	0.2012
Gizzard, %	1.29	1.22	1.22	1.25	0.9209

LBW: live body weight; CW: carcass weight; DP: dressing percentage; K-0: feed mixture without hemp addition; P-1: feed mixture with 1% hemp addition; P-2: feed mixture with 2% hemp addition; P-3: feed mixture with 3% hemp addition; Sig.: significance level; LSM with different superscripts in the same row significantly differ (Bonfferoni post hoc test, $P < 0.05$)

Table 5. The effect of dietary supplementation of the industrial hemp leaves on the pH values and color parameters (L*, a*, b*) of broiler breast meat (LSM values)

Trait (LSM)	K-0	P-1	P-2	P-3	*P*-value
			Treatment		
pH_1	6.15	6.07	6.05	6.10	0.7134
pH_2	5.80	5.79	5.81	5.81	0.9701
L* (lightness)	46.55^{ab}	46.24^{a}	48.28^{b}	46.56^{ab}	0.0295
a* (redness)	9.97	10.09	10.32	10.92	0.1653
b* (yellowness)	9.93	10.06	10.29	10.05	0.9081

pH_1: pH value measured 1 h *post mortem*; pH_2: pH value measured 24 h *post mortem*; K-0: feed mixture without hemp addition; P-1: feed mixture with 1% hemp addition; P-2: feed mixture with 2% hemp addition; P-3: feed mixture with 3% hemp addition; Sig.: significance level; LSM with different superscripts in the same row significantly differ (Bonfferoni post hoc test, $P < 0.05$)

Table 6. The effect of dietary supplementation of the industrial hemp leaves on drip loss (DL) values measured 24 hours (DL_24), 48 hours (DL_48), and 72 hours (DL_72) *post mortem*, thawing loss (TL), cooking loss (CL), and Warner-Bratzler shear force (WBSF) of broiler breast meat (LSM values)

Trait (LSM)	K-0	P-1	P-2	P-3	*P*-value
			Treatment		
DL_24, %	1.24	0.90	1.58	1.03	0.3875
DL_48, %	2.26	1.76	2.64	2.05	0.5185
DL_72, %	3.10	2.55	3.95	3.05	0.3221
TL, %	4.39	3.82	3.34	4.02	0.0794
CL, %	21.68	19.59	18.50	18.15	0.4576
WBSF, N	15.45^{ab}	17.22^{a}	13.47^{b}	14.37^{b}	0.0054

K-0: feed mixture without hemp addition; P-1: feed mixture with 1% hemp addition; P-2: feed mixture with 2% hemp addition; P-3: feed mixture with 3% hemp addition; LSM with different superscripts in the same row significantly differ (Bonfferoni post hoc test, $P < 0.05$)

The analysis of tenderness, i.e. WBSF, showed that the breast meat of group P-1 had the highest value (17.22 N) and was significantly different from groups P-2 (P = 0.0047) and P-3 (P = 0.0506; Table 6).

DISCUSSION

The present study showed that different amounts of industrial hemp leaves in the diet of broiler chicks had no significant effect on their average LBW (K-0 = 3035.4 g, P-1 = 3125.30 g, P-2 = 3077.90 g, P-3 = 2952.20 g; P = 0.467). Similarly, Tufarelli et al. (2023) found that hempseed intake at 5% (2.148 g) and 10% (2.155 g) had no significant effect on the average LBW of broiler chicks slaughtered at 49 days of age ($P > 0.05$). In contrast, Khan et al. (2010) reported that commercial broiler chicks slaughtered at 42 days of age had significantly higher LBW ($P < 0.05$) in the group supplemented with 20% hempseeds (2087.2 g) compared to the control group (1861.4 g). Khan et al. (2010) also found that the chicks in the group supplemented with 5% hempseeds had a lower LBW (1717.2 g), while the chicks in the group supplemented with 10% hempseeds had a higher LBW (1933.1 g), compared to the control group ($P < 0.05$). Šťastník et al. (2019) investigated the effects of adding hempseed expellers at levels of 5% and 15% to broiler diets. They found that a 15% addition of hempseed expeller reduced the LBW (2.079 g) of broilers at 37 days of age compared to the control diet (2.300 g), although CW was not significantly different.

No significant differences were found between the diets in terms of CW and DP ($P > 0.05$, Table 4). The mean DP reported in the technological procedure for Ross 308 (Aviagen Inc. Broiler Nutrition Specifications, 2022) is 73.42% at 3.000 g live weight. The present study shows that the mean DP value for the K-0 group was 77.02%, while the supplemented groups had DP values of 77.58% (P-1), 76.80% (P-2), and 76.84% (P-3). Similarly, studies on the dietary inclusion of hempseed (Khan et al., 2010) and hempseed expellers (Šťastník et al., 2019) also showed no significant differences in the DP values of broiler chicks. However, the DP values in the present study were higher than those reported by Khan et al. (2010) and Šťastník et al. (2019). Khan et al. (2010) found that the inclusion of hempseed in the diet at levels of 5%, 10%, and 20% resulted in DP values of 61.3%, 62.4%, and 63.3%, respectively. Šťastník et al. (2019) reported that including 5%hempseed expellers in the diet resulted in a DP of 72.56%, while a 15% hempseed expeller led to a DP of 70.70%. Researches on the inclusion of hemp leaves in the diet of broilers is still limited. While hempseed, hemp oil, and hemp cake have been extensively studied, their influences on the carcass final average LBW and DP are still controversial. The differences in results observed in aforementioned studies could be in possible differences in the digestibility of hemp supplements and their nutritional value.

The analysis of the individual carcass cuts showed that, aside from wing proportion, there were no significant differences between the experimental groups (Table 4). Compared to the results of the present study, the hybrid manual Ross 308 (Aviagen Inc. Broiler Nutrition Specifications, 2022) reported lower proportions of thigh (13.96%), breast (25.86%), and wing (7.50%), while the proportion of drumstick was higher (13.96%). Vispute et al. (2019) found that the addition of hempseed alone (0.25% and 0.3%) or in combination with dill seed (0.2% and 0.3%) had no significant effect on the individual proportion of breast, thigh, drumstick, wing, and neck in their carcasses ($P > 0.05$). Šťastník et al. (2016) found that the addition of 2.5% hempseed expellers and 1% pellets from technical hemp plant tops to the broiler feed had no significant effect on the proportion of breast (18.00% and 18.13%) and legs (14.40% and 15.14%) in the carcasses. Similarly, research by Šťastník et al. (2015) on the addition of 5% and 15% hempseed cakes in feed mixtures for broilers also showed no significant differences in the proportion of breast (21.33% and 19.41%) and legs (14.78% and 14.39%) in the carcasses.

The abdominal fat tissue of broilers shows faster growth compared to other fat tissues and serves as a reliable indicator of total body fat content (Białek et al., 2021). Fat deposition is considered unfavourable by producers and consumers alike, as it is perceived as a waste of dietary energy and an inferior by-product, which ultimately reduces carcass yield and affects consumer acceptance (Białek et al., 2021). In this study, the carcasses of the P-2 group (0.66%) and the P-3 group (0.59%) had a significantly lower proportion of abdominal fat than those of the P-1 group (1.06%). Eriksson and Wall (2012) found that chicks fed a diet containing 20% hempseed cake had a significantly lower proportion of abdominal fat (1.36%) than chicks fed a diet containing soybean cake, rapeseed cake, and peas (1.74%; $P < 0.01$). Contrary, Tufarelli et al. (2023) found that there was no significant difference in the proportion of abdominal fat between chicks fed a control diet (1.8%) and those supplemented with dietary inclusion of hempseed cake at 5% (1.9%) and 10% (2.1%) levels ($P > 0.05$). Similarly, Vispute et al. (2019) found that supplementing the diet with hempseed alone at 0.2% (1.97%) and 0.3% (1.62%) or in combination with the dill seed at 0.2% (1.91%) and 0.3% (1.58%) had no significant effect on the proportion of abdominal fat.

The proportions of heart, liver, and gizzard showed no significant differences between the experimental groups ($P > 0.05$; Table 4). Bień et al. (2024) also found no significant differences in the proportion of liver in the carcasses of broilers supplemented with hemp extract (3% of the feed on top). In a study by Konca et al. (2014), it was observed that a 10% addition of hempseed to the diet led to an increase in heart weight but a decrease in liver and intestinal weight in quails during the fattening period, with no effect on gizzard weight. Vispute et al. (2019) also found that the addition of hempseed alone (0.2% and 0.3%) or in combination with dill seed (0.2% and 0.3%) had no significant effect on heart, liver, and gizzard weights, as well as total giblet organ weights.

The pH value of broiler meat is an important quality trait, that affects several important characteristics such as color, water-holding capacity, tenderness, juiciness, and shelf life (Mir et al., 2017). The final pH value in broiler breast muscles was between 5.6 and 5.9, while the final pH value in thigh muscles was higher, ranging from 6.1 to 6.4 (Kralik and Kralik, 2023). The pH values of the breast meat in the present study were within this range (Table 5) and were not affected by the addition of industrial hemp leaves. Consistent with this, studies using hempseed oil at 25%, 50%, and 100% in broiler diets (Kanbur, 2022), hempseed expeller at 5% and 15% in broiler diets (Šťastník et al., 2019) or hempseed cake at 5% and 10% (Tufarelli et al., 2023) found no significant differences in the pH value of breast meat as well.

Meat color is an important trait for the meat industry, as it strongly determines the consumers' perception of product quality and thus significantly influences the purchase decision (Ruedt et al., 2023). Qiao et al. (2001) classified chicken breast muscle into three groups based on color: "lighter than normal" (L* > 53), "normal" (48 < L* > 53) and "darker than normal" (L* < 48). The comparison of our results with these criteria showed that breast muscles from the group P-2 (48.28) were "normal", while others (K-0 = 46.55, P-1 = 46.24, and P-3 = 46.56) were slightly lower and could be categorized as "darker than normal". However, it should be noted that consumers easily accept darker meat with lower L* and higher a* and b* than, for example, meat that is significantly paler or discolored with white stripes (Kuttappan et al., 2012). Therefore, this slightly darker-colored breast muscle should not be a key factor in the purchasing decision. Kanbur (2022) investigated the effects of using increasing amounts of hempseed oil instead of soybean oil in broiler diets and found that the hempseed oil in the diet did not affect L* or a* color values regardless the amounts added (25%, 50%, and 100%), while adding 100% hempseed oil to the diet resulted in a significantly lower b* value in the meat (P < 0.05). Šťastník et al. (2019) found that the addition of hempseed expeller up to 15% did not affect the L* color value of broiler meat, while the a* and b* values were significantly higher (P < 0.05) compared to the experimental groups (0% and 5% of hempseed expellers). Kaić et al. (2024) found that the addition of industrial hemp leaves at 1% only, affected the b* color parameter of the broiler's breast muscle, which was significantly higher (P < 0.05) than in other groups (0%, 2%, and 3% hemp leaves). Contrary, Tufarelli et al. (2023) found that the inclusion of hempseed cake at 5% and 10% levels did not significantly affect the L*, a*, and b* values of broilers' breast meat (P > 0.05).

The quality of poultry meat is largely determined by the WHC, which plays a decisive role in processing technology and consumer acceptance. Excessive dripping of meat and meat products, due to poor WHC, can lead to significant weight loss of carcasses and cuts. This could have a negative impact on the yield and quality of fresh and processed meat, making it less attractive to consumers (Warner, 2017). It has been shown that DL, as a measure of WHC, is one of the most important parameters for meat quality assessment (Torres Filho et al., 2017). Similar to the present study, Kanbur (2022) found that the substitution of soybean oil with hempseed oil at increasing levels in broiler diets had no effect on the WHC of breast meat, regardless of the level of supplementation (25%, 50%, and 100% hempseed oil). In contrast, Bień et al. (2024) found that the breast muscle of broilers fed 3.0% hemp extract in the diet had a 0.05% lower DL value after 24 hours than control group. It is also important to note that exudation is a gradual process in which water is expelled from the myofibrils and drains from the muscle over time (Den Hertog-Meischke et al., 1997). This is in line with the results of our study and with literature reports on different types of meat (Correa et al., 2007; Razmaitė et al., 2017; Torres Filho et al., 2017), which show a positive linear relationship between the measurement interval and DL as well.

Freezing and subsequent thawing of meat can significantly affect its quality characteristics, such as moisture retention, protein denaturation, oxidation, color, and texture, ultimately reducing consumer acceptance (Leygonie et al., 2012). The TL found in this study are in agreement with the previous one (Kaić et al., 2024), suggesting that the inclusion of 1%, 2%, and 3% hemp leaves in the diet has no effect on the TL of broiler breast muscles. In the present study, the TL value for group K-0 was 4.39%, while the TL values for groups P-1, P-2, and P-3 were 3.82%, 3.34%, and 4.02%, respectively. However, comparing the TL values of different studies, our previous study (Kaić et al., 2024) showed higher TL values for the control group 5.00%, and supplemented with 1%, 2%, and 3% hemp leaves, the TL values were 4.95%, 5.57%, and 5.94%, respectively. Yalcin et al. (2018) studied the effect of hempseed supplementation (5%, 10%, and 20%) on the meat quality of Japanese quails. The group with a 20% hempseed supplement had the lowest TL value (2.56%), while the groups with 5% and 10% hempseed supplement had similar TL values (4.76% and 4.48%, respectively) as in Kaić et al. (2024). Yalcin et al. (2018) reported that 20% hempseed supplementation reduced TL and thereby improved meat quality, although the difference was not statistically significant.

The cooking yield provides information on the properties of the raw muscle protein and the functionality of the meat, which has a direct impact on the yield and quality of the further-processed meat products (Li et al., 2013). Consequently, CL provides essential insights into the factors influencing meat quality and helps to predict properties during further processing. The non-significant differences in CL observed in this study are in agreement with our previous one (Kaić et al., 2024), suggesting that the inclusion of 1%, 2%, and 3% hemp leaves in the diet has no effect on the CL of their breast muscles. In the present study, the CL for the K-0 group was 21.68%, while for the hemp leaf supplemented groups was 19.59% (P-1), 18.50% (P-2), and 18.15% (P-3). Although the differences in CL between groups were not significant ($P > 0.05$), it should be noted that hemp leaf supplementation resulted in a lower CL compared to the control group. However, when comparing the CL values of different studies, earlier research shows lower CL values. Kaić et al. (2024) reported a CL of 16.67% for the control group and 18.11%, 17.60%, and 18.37% for the groups supplemented with 1%, 2%, and 3% hemp leaves, respectively. In a study with Japanese quails, Yalcin et al. (2018) found that supplementing with 20% hempseed resulted in a significant decrease in CL (12.84%), which, together with the above-mentioned reduction in TL, improved meat quality.

In addition to the meat quality, tenderness is another important and complex quality trait that influences consumer acceptance. In particular, consumers can recognize differences in the tenderness of meat and are willing to pay a higher price if they perceive the meat to be more tender (Polkinghorne and Thompson, 2010). The present study shows that the WBSF value for the K-0 group was 15.45 N, while the supplemented groups had WBSF values of 17.22 N (P-1), 13.47 N (P-2), and 14.37 N (P-3). In contrast, studies on the dietary inclusion of hemp extracts (Bień et al., 2024) and hempseed expellers (Šťastník et al., 2019) found no significant differences in breast meat tenderness. However, the WBSF values in the present study were lower than those reported by Bień et al. (2024) and higher than those reported by Šťastník et al. (2019). Bień et al. (2024) found that the addition of hemp extract to the diet (3% of the total diet) resulted in WBSF values of 38.01 N, while the control group had WBSF values of 41.54 N. Šťastník et al. (2019) reported that the breast muscles of chicks fed a diet without hempseed expellers had a WBSF of 11.23 N, while the breast muscles of chicks fed 5% and 15% hempseed expellers had WBSF values of 10.74 N and 11.38 N, respectively. These results clearly show the different effects of the various hemp by-products on meat tenderness, highlighting differences observed across various studies. Furthermore, Schilling et al. (2003) found a correlation between the instrumental and sensory acceptability of chicken breast tenderness. They found that consumers perceived samples with a shear force value between 1.1 kg/cm^2 and 3.1 kg/cm^2 as tender and highly acceptable, while higher values indicated increased toughness and lower acceptability. The results of our study show that broiler breast meat remains tender regardless of the addition of hemp leaves and should be therefore highly accepted by consumers.

CONCLUSIONS

Supplementation with industrial hemp leaves affects certain carcass and meat quality traits in broilers. In particular, 2% supplementation resulted in the highest wing proportion and the lightest breast meat color. Both the 2% and 3% supplementation groups had significantly lower abdominal fat yield compared to the 1% supplementation group. The results of the Warner-Bratzler shear force (WBSF) showed that the 1% supplementation group had the highest WBSF, indicating potentially tougher meat compared to the 2% and 3% supplementation groups. However, no significant differences were found for other meat quality traits. These results suggest that hemp leaves could be a valuable feed supplement to improve certain aspects of broiler meat quality, such as reducing abdominal fat and potentially influencing meat color and tenderness. Further research is recommended to fully understand the effects of hemp leaves on other meat quality traits and broiler health before it is widely used in poultry production.

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