Italian Journal of Animal Science

ISSN: 1828-051X (Online) Journal homepage: www.tandfonline.com/journals/tjas20

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To cite this article: Yusup Sopian, Katatikarn Sahatsanon, Apinya Satsook, Chaiwat Arjin, Chompunut Lumsangkul, Korawan Sringarm, Panneepa Sivapirunthep, Awis Qurni Sazili & Chanporn Chaosap (2025) Effect of *Cannabis sativa* residue supplementation on meat quality, antioxidative capacity, and immune response in 34-day-old broiler chickens, Italian Journal of Animal Science, 24:1, 1294-1306, DOI: 10.1080/1828051X.2025.2511270

To link to this article: https://doi.org/10.1080/1828051X.2025.2511270

Published online: 03 Jun 2025.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2025, VOL. 24, NO. 1, 1294–1306
https://doi.org/10.1080/1828051X.2025.2511270

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RESEARCH ARTICLE

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Effect of *Cannabis sativa* residue supplementation on meat quality, antioxidative capacity, and immune response in 34-day-old broiler chickens

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ABSTRACT

The overuse of antibiotics in animal feed has led to rising antibiotic resistance, highlighting the need for sustainable alternatives like herbal supplements. *Cannabis sativa* residues (CR), byproducts of the cannabis industry, have medicinal properties that may benefit poultry production. This study evaluated the effects of CR supplementation on meat quality, antioxidative capacity, and immune response in 34-day-old broiler chickens. A total of 256 male one-day-old Ross 308 chicks were randomly divided into four groups (0%, 0.5%, 1%, or 2% CR) and reared for 34 days. Broilers fed 1% CR had the highest eviscerated carcase yield, while those receiving 2% CR had the highest breast and intestine percentages ($p < 0.05$). A 2% CR dietary inclusion significantly elevated the pH of breast meat at 24 h post-mortem and reduced shear force and drip loss in breast meat ($p < 0.05$). Malondialdehyde levels in meat were significantly lower during storage (0, 3, and 5 days) in CR-fed groups ($p < 0.01$). No significant changes were observed in thawing loss, cooking loss, or meat colour ($p > 0.05$). While CR had no significant effect on immune-related gene expression, it significantly upregulated antioxidant-related genes such as catalase (CAT) and nuclear factor erythroid 2–related factor 2 (Nrf2) in the liver and increased Nrf2 expression in the jejunum ($p < 0.05$). Principal component analysis revealed positive correlations between CR supplementation and antioxidant gene expression. These results suggest that CR, at 0.5 to 2%, may enhance meat quality and antioxidant defense in broiler chickens.

HIGHLIGHTS

- *Cannabis sativa* residues improved meat pH and reduced drip loss and shear force.
- *Cannabis sativa* residues reduced malondialdehyde content and enhanced meat quality.
- *Cannabis sativa* residues up-regulated antioxidant-related genes.

ARTICLE HISTORY
Received 24 March 2025
Revised 2 May 2025
Accepted 20 May 2025

KEYWORDS
Carcase; drip loss; malondialdehyde; immune-related gene

Introduction

Antibiotic growth promoters (AGPs) have been widely used in animal feed for decades to enhance productivity and prevent disease in various animal species (Ronquillo and Hernandez 2017). However, the extensive use of AGPs has raised significant public health concerns due to the development of antibiotic resistance (Muaz et al. 2018). Consequently, the European Union banned AGPs in 2006, and many other countries are considering similar measures because of the potential risks posed by AGP residues in meat (Muaz et al. 2018). These concerns have driven increased research into alternative solutions to replace AGPs in broiler diets (Mashayekhi et al. 2018; Zaker-Esteghamati et al. 2021).

Recently, natural additives derived from medicinal herbs have attracted interest as potential alternatives to AGPs, owing to their capacity to enhance broiler performance, meat quality, and carcase traits (Mashayekhi et al. 2018; Foroutankhah et al. 2019). Moreover, these herbs may function as natural antioxidants, which are often preferred by consumers over

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synthetic alternatives (Salami et al. 2016). *Cannabis sativa* has been used for its medicinal properties for centuries, but its use declined in the early twentieth century after being classified as a controlled substance due to its psychoactive effects (Balant et al. 2021). The recent legalisation of *C. sativa* in various countries, including Thailand in 2019, has reignited interest in its medicinal and recreational uses (Zinboonyahgoon et al. 2021). Thailand was the first Southeast Asian nation to legalise *C. sativa* for medical and research purposes, initially granting permission to eight institutions to cultivate and process the plant for medical-grade products (Somwaiya and Saardphak 2019). As a result, there has been a rapid increase in the number of licenced producers (Kalayasiri and Boonthae 2023).

Cannabis sativa products are typically derived from the inflorescences, with the other parts left behind as residues. These residues contain valuable bioactive compounds, including cannabidiol (CBD), tetrahydrocannabinol (THC), terpenes, and flavonoids, which may offer medicinal benefits (Lewis et al. 2017; Fordjour et al. 2023). Recent studies have shown that *C. sativa* biomass could enhance antioxidant capacity in animals and improve immune function (Parker et al. 2022; Wang K et al. 2023), without negatively impacting productivity or carcase traits in sheep and goats (Krebs et al. 2021; Khamhan et al. 2023). In broilers, earlier studies have demonstrated that *C. sativa* extract supplementation in chicken diets helps maintain body weight under stress-induced conditions (Konieczka et al. 2020; Bień et al. 2024), and the plant also exhibiting anti-inflammatory, antioxidant, and antimicrobial properties (Marsh and Smid 2020; Atalay et al. 2021; Fordjour et al. 2023). Despite these promising effects, research on the use of *C. sativa* residues (CR) in poultry feed remains limited.

In our earlier research, we found that supplementing broiler chicken diets with CR for up to 40 days improved plasma antioxidant status, intestinal structure, and breast meat free amino acid content and fatty acid profile without negatively affecting growth, carcase traits, or meat quality (Sopian et al. 2024; Sopian et al. 2025). However, the efficacy of plant-based feed additives in broilers may vary with age (Pascual et al. 2022; Duangnumsawang et al. 2023). Moreover, age-related differences in immune responses to pathogens have been documented, highlighting the importance of considering developmental stages in poultry research (Gaunson et al. 2006). Additionally, the practical slaughter age for broilers in Thailand typically ranges from 32 to 42 days (Prachantasena et al. 2016). Therefore, this study aimed to evaluate the potential of CR as a feed additive by assessing its effects on carcase traits, meat quality, antioxidative capacity, and immunity response in broilers at 34 days of age.

Material and methods

Animals and experimental design

This experiment was approved by the ethical committee of the Faculty of Agriculture, Chiang Mai University (Approval No. AGIACUC015/2665). A total of 256 one-day-old male Ross 308 broiler chicks were obtained from Charoen Pokphand Company Limited (CP), Bangkok, Thailand. The chicks were randomly assigned to four groups (64 chickens/group) with eight replication (8 chickens/replication). They were housed in an evaporative cooling system with 32 floor pens (120 cm × 100 cm × 50 cm). The chicks had unrestricted access to feed and water throughout the study period. The lighting regimen consisted of continuous illumination during the first week, followed by a 16-h light/8-h dark cycle using incandescent yellow lights at an intensity of 10 lux.

The experimental diets were crumbled commercial diets (CP, Bangkok, Thailand) formulated to meet broiler nutritional requirements based on NRC (1994) guidelines. The four dietary treatments included a control group (CON) receiving a basal diet and three experimental groups supplemented with 0.5%, 1%, or 2% CR. The CR, consisting of air-dried *C. sativa* leaves and stalks, was obtained from the Pethlanna joint venture in Lampang, Thailand. It was oven-dried at 60 °C for 4 h until brittle, then ground into a fine powder using a hammer mill (Retsch SM100, Germany). The details of CR preparation and its chemical composition were reported in our previous study (Sopian et al. 2024). The CR contained 91.73% dry matter, 13.34% crude protein, 4.21% ether extract, 61.68% neutral detergent fibre, and 42.76% acid detergent fibre (Sopian et al. 2024). All chicks were initially provided with the control diet for a two-day adaptation period before transitioning to their respective experimental diets. The proximate composition and cannabinoid content of the diets were analysed following AOAC (2005) standards. For feed preparation, CR was supplemented on top of the basal diets at inclusion rates of 0.5, 1, and 2 g per 100 g of feed, with diets prepared in three separate batches during each phase (starter and finisher). Feed samples were randomly collected from each batch and subjected to proximate analysis. Despite the addition of CR, the proximate analysis conducted in triplicate revealed no significant

Table 1. Measured chemical composition (g/kg) experimental diets in the starter and finisher diet supplemented with *Cannabis sativa* residue (CR).

	Starter (1–23 days)				Finisher (24–34 days)			
Variables[a]	0%CR	0.5%CR	1%CR	2%CR	0%CR	0.5%CR	1%CR	2%CR
DM	915.7	914.5	911.8	913.3	909.0	908.2	908.8	909.1
CP	220.8	210.6	216.3	221.8	217.6	207.0	202.5	215.3
EE	53.1	61.6	56.9	53.1	55.9	45.0	47.4	47.4
Ash	62.2	65.9	67.6	68.6	57.5	54.7	54.6	59.2
CF	35.3	35.0	36.1	34.6	32.2	33.2	29.9	32.1
GE (MJ/kg)	16.71	16.29	16.67	16.42	16.50	16.30	16.18	16.01

[a]DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fibre; GE = gross energy.

Table 2. Cannabinoid composition (ppm) experimental diets in the starter and finisher diet supplemented with *Cannabis sativa* residue (CR).

	Starter (1–23 days)				Finisher (24–34 days)			
Variables[a]	0%CR	0.5%CR	1%CR	2%CR	0%CR	0.5%CR	1%CR	2%CR
CBDA	nd	3.26	3.28	3.26	nd	3.25	3.25	3.42
CBD	nd	1.59	2.38	2.87	nd	1.24	1.30	4.84
CBN	nd	2.14	5.09	9.36	nd	3.55	6.79	13.12
THC	nd	8.64	18.06	30.66	nd	7.98	18.01	37.57
THCA	nd	3.19	7.29	8.81	nd	1.69	5.09	15.29

[a]CBDA = Cannabidiolic acid; CBD = Cannabidiol; CBN = cannabinol; THC = Delta-9-tetrahydrocannabinol; THCA = Tetrahydrocannabinolic acid. nd: not detected.

differences ($p > 0.05$) in the major nutritional components (dry matter, crude protein, ether extract, crude fibre, ash, and gross energy) among the treatment groups. The detailed nutritional composition of experimental diets is presented in Tables 1 and 2.

Data collection

Sample collection

The body weight (BW) of the broilers was recorded at the beginning and end of the trial. On day 34, after overnight feed withdrawal, two birds per replicate ($n = 16$ per treatment) were randomly chosen for slaughter *via* cervical dislocation. The carcases were scalded at 60 °C for three minutes, de-feathered, and manually eviscerated, then chilled in an ice bath for 45 min. Liver and jejunum samples were collected from one bird per replicate ($n = 8$ per treatment), immediately preserved in RNAlater, and frozen at −80 °C for subsequent gene expression analysis.

Slaughter weight (SW) and eviscerated carcase (EC) weights were recorded, with the visceral organs expressed as percentages of SW. Carcase cuts were calculated as a proportion of EC. Breast and thigh muscles were harvested for meat quality assessment. The pH of the left-side breast and thigh muscles was measured at 3 h post-mortem, followed by storage at 4 °C for 24 h to determine colour and ultimate pH (pH_{24}). These samples were then weighed and stored at −20 °C for further analysis, including thawing loss, cooking loss, and shear force. The right-side breast and thigh muscles were reserved for drip loss measurement, with the breast muscle further analysed for antioxidative stability.

Meat quality and oxidative stability

The meat quality parameters were measured according to Chaosap et al. (2020). The pH of the left-side thigh and breast muscles was measured at 3- and 24-h post-mortem using SG2-ELK Seven Go™ pH metre (Mettler Toledo, Shanghai, China). Meat colour was evaluated 24 h post-mortem at three distinct locations on the meat surface using a model CM-5 spectrophotometer (Konica Minolta, Osaka, Japan). The colour was assessed based on the CIE colour parameters: lightness (L*), redness (a*), and yellowness (b*) using a d/8° viewing geometry, an 8 mm aperture diameter, and a standard D65 illuminant. Drip loss was determined by weighing the meat samples, sealing them in plastic bags, and suspending them on hooks at 4 °C for 24 h. The samples were then blotted dry with paper towels and reweighed. The thawing loss was assessed by freezing samples at −20 °C for seven days, followed by a 12-h thawing period at 4 °C, with the weight difference calculated before and after thawing. For cooking loss and texture analysis, samples were submerged in a water bath at 80 °C (Memmert, Buchenbach, Germany) until their internal temperature reached 70 °C, requiring approximately 20 min. Following this, the samples were cooled under

running tap water until reaching room temperature, reweighed, and cut into eight uniform sections (1 × 2 × 1 cm), ensuring alignment with muscle fibres. The shear force of these samples was measured using a Texture Analyser (TA.XT Plus, Texture Technologies Corp., Surrey, UK). The thiobarbituric acid reactive substances (TBARS) assay was performed in breast meat samples stored at 4 °C for 0, 3, and 5 days, following the method described by Sringarm et al. (2022). TBARS levels were analysed using Agilent 1220 Infinity II high-performance liquid chromatography system (Agilent Technologies, Santa Clara, USA). Results were expressed as milligrams of malondialdehyde (MDA) per kilogram of sample.

Antioxidant and immune-related gene expression

Total RNA was extracted from liver and jejunum tissues using the PureLink™ RNA Mini Kit (Invitrogen, USA). The concentration and purity of the RNA were evaluated using a NanoDrop® ND-2000c Spectrophotometer (Thermo Scientific, USA). The RNA was then reverse transcribed into complementary DNA (cDNA) with BioRad's iScript™ cDNA Synthesis Kit. Quantitative real-time PCR (qPCR) was performed using BioRad's CFX Connect™ Real-Time PCR System and iTaq Universal SYBR Green Supermix, following the manufacturer's instructions. The primer sequences used for the analysis are listed in Table 3. Gene expression was normalised to β-actin as the reference gene, and relative mRNA expression was determined using $2^{-\Delta\Delta}$Ct method (Livak and Schmittgen 2001).

Table 3. Primer sequences of the reference and target genes for real-time RT-PCR.

Target gene[1]		Primer sequences	Product size (bp)
Housekeeping gene			
ß-Actin	Forward	CTGGCACCTAGCACAATGAA	109
	Reverse	ACATCTGCTGGAAGGTGGAC	
Immune-related gene			
IL-1ß	Forward	GTGAGGCTCAACATTGCGCTGTA	214
	Reverse	TGTCCAGGCGGTAGAAGATGAAG	
IL-10	Forward	AGCAGATCAAGGAGACGTTC	103
	Reverse	ATCAGCAGGTACTCCTCGAT	
TNF-α	Forward	TGCTGTTCTATGACCGCC	174
	Reverse	CTTTCAGAGCATCAACGCA	
IFN-γ	Forward	CTGAAGAACTGGACAGAGAG	264
	Reverse	CACCAGCTTCTGTAAGATGC	
Antioxidants-related gene			
SOD	Forward	GCCACCTACGTGAACAACCT	208
	Reverse	AGTCACGTTTGATGGCTTCC	
CAT	Forward	CCACGTGGACCTCTTCTTGT	165
	Reverse	AAACACTTTCGCCTTGCAGT	
GPX	Forward	CAGCAAGAACCAGACACCAA	156
	Reverse	CCAGGTTGGTTCTTCTCCAG	
Nrf2	Forward	CAGAAGCTTTCCCGTTCATAGA	120
	Reverse	TGGGTGGCTGAGTTTGATTAG	

[1]ß-actin = beta actin IL-1ß = interleukin 1 beta; IL-10= interleukin 10; TNF-α = tumour necrosis factor alpha; IFN-γ = interferon gamma; SOD = superoxide dismutase; CAT = catalase; GPX = glutathione peroxidase; Nrf2 = nuclear factor erythroid-2-related factor 2.

Statistical analysis

The study followed a completely randomised design, with dietary treatments (0%, 0.5%, 1%, and 2% CR) treated as fixed effects. Data analysis was conducted using the PROC GLM procedure in SAS (SAS Institute, Cary, NC, USA), except for TBARS data, which was analysed through repeated-measures ANOVA in SAS. The model incorporated dietary treatment, storage duration (0, 3, and 5 days), and their interaction as fixed effects. Mean comparisons were performed using the PDIFF option in SAS, with statistical significance set at $p < 0.05$. Each parameter was analysed at the individual bird level, with two randomly selected birds per pen used to assess carcase traits, organ weights, meat quality, and TBARS values, while one bird per pen was designated for gene expression analysis. Additionally, principal component analysis (PCA) of the measured parameters was carried out using XLSTAT software (Addinsoft, New York, USA).

Results

Carcase traits and internal organs

Table 4 presents the impact of CR supplementation on broiler carcase traits and the proportion of visceral organ weights. Dietary CR in the diet did not significantly influence slaughter weight or the percentage of the thigh, wing, back, drumstick, feet, or neck ($p > 0.05$). However, CR supplementation significantly influenced the proportion of EC ($p = 0.002$), breast

Table 4. Effect of *Cannabis sativa* residue (CR) supplementation on slaughter weight, eviscerated carcase, and visceral organs in broiler chickens at 34 days of age.

	Treatments					
Variables[1]	Control	0.5% CR	1% CR	2% CR	SEM	*p*-Value
IBW (g)	63.98	63.23	64.76	64.38	0.22	0.081
SW (kg)	1.38	1.34	1.38	1.39	0.01	0.093
EC (% SW)	83.34^{A,B}	83.94^{A}	84.12^{A}	81.34^{B}	0.30	0.002
Carcase yield (% EC)						
Breast	24.64^{ab}	23.98^{b}	25.35^{ab}	25.81^{a}	0.23	0.027
Thigh	12.31	12.68	12.84	12.46	0.17	0.724
Wing	9.25	9.13	9.47	9.13	0.08	0.335
Back	26.11	25.83	24.77	26.44	0.25	0.087
Drumstick	12.16	12.58	11.84	12.53	0.11	0.058
Feet	5.09	4.90	4.97	5.03	0.04	0.399
Head	3.78^{A}	3.37^{B}	3.25^{B}	3.50^{A,B}	0.05	0.002
Neck	5.41	5.25	5.56	5.06	0.11	0.446
Visceral organs (% SW)						
Liver	2.43	2.57	2.39	2.37	0.03	0.109
Gizzard	3.20	3.37	3.21	3.13	0.06	0.561
Heart	0.49	0.49	0.48	0.47	0.01	0.650
Spleen	0.12	0.12	0.10	0.11	0.01	0.513
Intestine	5.63^{ab}	5.76^{ab}	5.16^{b}	5.94^{a}	0.09	0.018

[1]IBW = initial body weight; SW = slaughter weight; EC = eviscerated carcase. ^{A,B}LSMeans with different superscript letters within rows are highly significantly different ($p < 0.01$). ^{a,b}LSMeans with different superscript letters within rows are significantly different ($p < 0.05$).

($p = 0.027$), and head ($p = 0.002$). Broilers receiving a 1% CR-supplemented diet demonstrated the highest EC percentage, whereas those fed 2% CR had the greatest breast and intestine proportions ($p = 0.018$) but the lowest EC among the CR-fed groups. The proportions of other visceral organs (spleen, liver, gizzard, and heart) did not differ significantly ($p > 0.05$).

Table 5. Breast and thigh meat quality[a] in broiler chickens supplemented with *Cannabis sativa* residue (CR) at 34 days of age.

	Treatments					
Variables[b]	Control	0.5% CR	1% CR	2% CR	SEM	*p*-Value
Breast meat						
pH_3	6.33^{ab}	6.33^{ab}	6.43^{a}	6.24^{b}	0.022	0.026
pH_{24}	5.96^{B}	5.96^{B}	6.07^{AB}	6.18^{A}	0.024	0.003
Lightness (L*)	52.53	54.79	54.09	52.41	0.396	0.084
Redness (a*)	−1.17	−0.93	−1.08	−1.33	0.084	0.418
Yellowness (b*)	6.86	7.85	6.79	6.35	0.229	0.123
Drip loss (%)	3.15^{ab}	3.45^{a}	3.22^{ab}	2.60^{b}	0.096	0.011
Thawing loss (%)	8.85	9.21	8.37	7.46	0.290	0.166
Cooking loss (%)	10.71	11.04	11.78	10.76	0.368	0.723
Shear force (kg)	1.84^{A}	1.88^{A}	1.66^{AB}	1.42^{B}	0.035	0.001
Thigh meat						
pH_3	6.61	6.65	6.60	6.60	0.017	0.682
pH_{24}	6.54	6.50	6.48	6.48	0.019	0.695
Lightness (L*)	55.27	56.12	56.77	55.44	0.377	0.487
Redness (a*)	1.63	2.10	2.12	1.30	0.177	0.291
Yellowness (b*)	3.48	4.43	4.14	4.76	0.281	0.432
Drip loss (%)	3.20^{AB}	3.53^{A}	3.18^{AB}	2.54^{B}	0.101	0.004
Thawing loss (%)	2.24	2.15	2.26	2.17	0.149	0.993
Cooking loss (%)	7.36	8.59	9.08	8.76	0.304	0.206
Shear force (kg)	0.99	1.02	0.99	0.95	0.020	0.712

[a]Data represents mean values of 16 replicate per treatment; Muscle pH was measured at 3- and 24-h post-mortem. [A,B]LSMeans with different superscript letters within rows are highly significantly different ($p < 0.01$). [a,b]LSMeans with different superscript letters within rows are significantly different ($p < 0.05$).

Meat quality

Table 5 shows the impact of dietary CR supplementation on meat quality. The ultimate pH in the 2% CR group was significantly higher than control ($p = 0.003$). In addition, the inclusion of 2% CR in the diet significantly reduced both shear force ($p = 0.001$) and drip loss in breast meat ($p = 0.011$). For thigh meat, the lowest drip loss was observed in the 2% CR group ($p = 0.004$). However, CR supplementation did not significantly affect the lightness, redness, yellowness, or thawing loss of either breast or thigh meat ($p > 0.05$).

Meat oxidative stability

A notable interaction between dietary treatment and storage duration was observed in TBARS values ($p = 0.001$). Regardless of storage time, the control group consistently displayed higher TBARS levels compared to the CR-supplemented groups. Additionally, TBARS values increased with storage time for all treatment groups (Figure 1). On days 0 and 3, there were no significant differences in TBARS levels between the CR-supplemented groups (0.5%, 1%, and 2% CR). However, by day 5, the 0.5% CR group had higher TBARS values than the 1% and 2% CR groups.

Genes expression

Figure 2 illustrates the effects of CR supplementation on antioxidant and immune-related gene expression in the liver and jejunum. Supplementation with CR significantly increased the Nrf2 ($p = 0.018$) and CAT genes

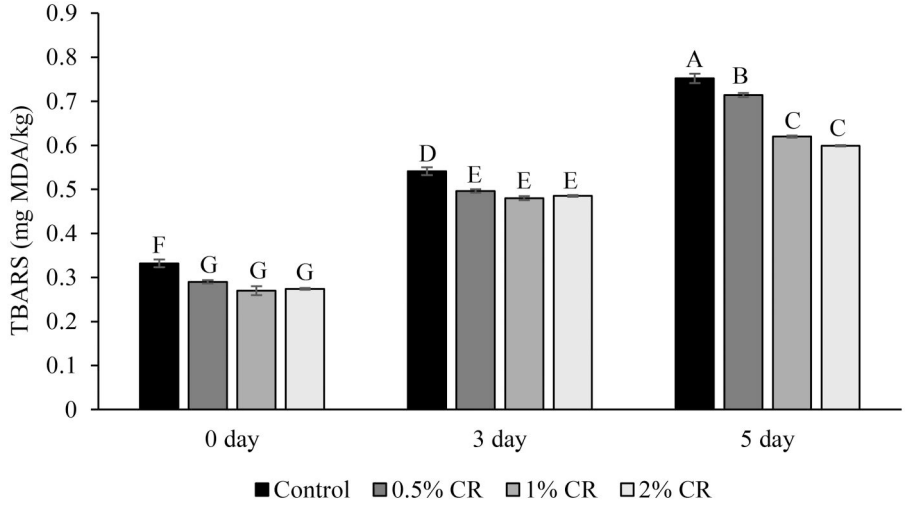

Figure 1. Effect of *Cannabis sativa* residue (CR) supplementation in broiler chicken diets on breast meat oxidative stability, measured as malondialdehyde (MDA) levels. Treatments include 0.5% CR, 1% CR, and 2% CR added to the diet. LSMeans with different superscript letters (A-G) differ significantly ($p = 0.001$). Thiobarbituric acid reactive substances (TBARS).

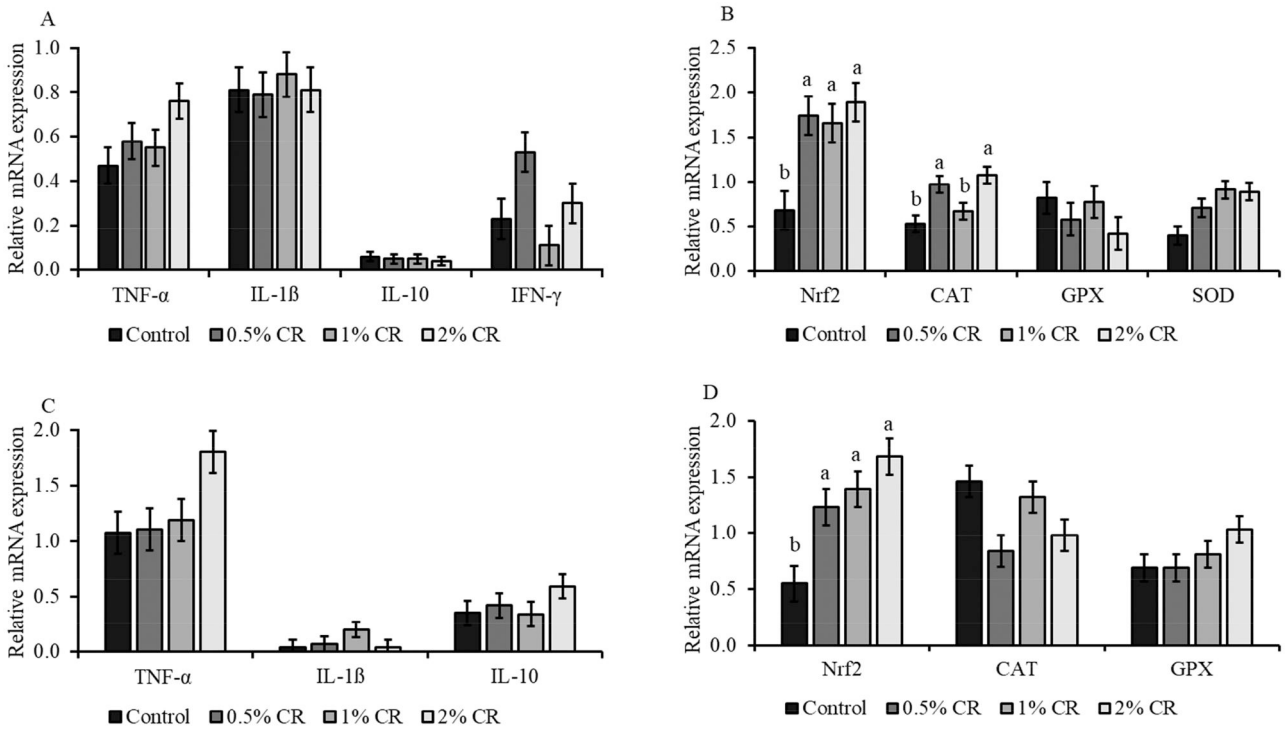

Figure 2. Effect of *Cannabis sativa* residue (CR) supplementation in broiler chicken diets on antioxidant and anti-inflammatory related genes expression. Treatments include 0.5% CR, 1% CR, and 2% CR added to the diet. Data are presented as LSMeans ± standard error of the mean (SEM). Relative expression of immune related genes (A) and antioxidant genes expression (B) in the liver. Relative expression of immune related genes (C) and antioxidant genes (D) in the jejunum. Nuclear factor erythroid 2–related factor 2 (Nrf2), catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD), tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1ß), interleukin 10 (IL-10), interferon gamma (IFN-γ). Relative mRNA expression levels were normalised to β-actin as the reference gene. Values represent means ± SEM. Bars with different letters indicate significant differences between groups ($p < 0.05$).

in the liver ($p = 0.017$). Similarly, all CR-treated groups showed elevated Nrf2 expression in the jejunum ($p = 0.025$). However, no significant differences were observed in the expression of GPX and SOD in the liver ($p > 0.05$), nor in CAT and GPX expression in the jejunum ($p > 0.05$). Furthermore, dietary CR had no significant effect on immune-related gene markers, including IL-1β, IFN-γ, IL-10, and TNF-α ($p > 0.05$).

Principal component analysis

The PCA results show that the first two principal components (PC1 and PC2) explain 50.83% and 35.49% of the variance in the data set, accounting for 86.33% of the total variability (Figure 3). PC1, which relates to antioxidant properties, showed that TBARS levels were on the right side of the biplot at all storage times, while Nrf2 expression in the jejunum (Nrf2_J) and liver (Nrf2_L) and CAT expression in the liver (CAT_L) were on the opposite side. PC2, related to meat physical properties, showed that shear force, pH_3, and drip loss were on the opposite side of pH_{24}. Biplot analysis revealed that CR supplementation was positively related to Nrf2_J and Nrf2_L and CAT_L at all levels, while the control group was positively associated with TBARS across all storage times. In terms of physical properties, the 2% CR supplement was closely and positively related to pH_{24}, but negatively related to pH_3, drip loss, and shear force, which were instead positively related to the control group, 0.5% CR and 1% CR treatments. This analysis highlights the different relationships between CR supplementation levels, antioxidant markers and meat quality parameters.

Discussion

The results indicate that most carcase traits were unaffected by the different experimental treatments. No significant differences were found in carcase characteristics among the treatments, except broilers receiving a 1% CR-supplemented diet showed the highest EC percentage, whereas those fed 2% CR had the highest breast and intestine proportions but the lowest EC among the CR-fed groups. The higher EC observed in the 1% CR group could indicate an enhanced energy reserve or improved cellular function

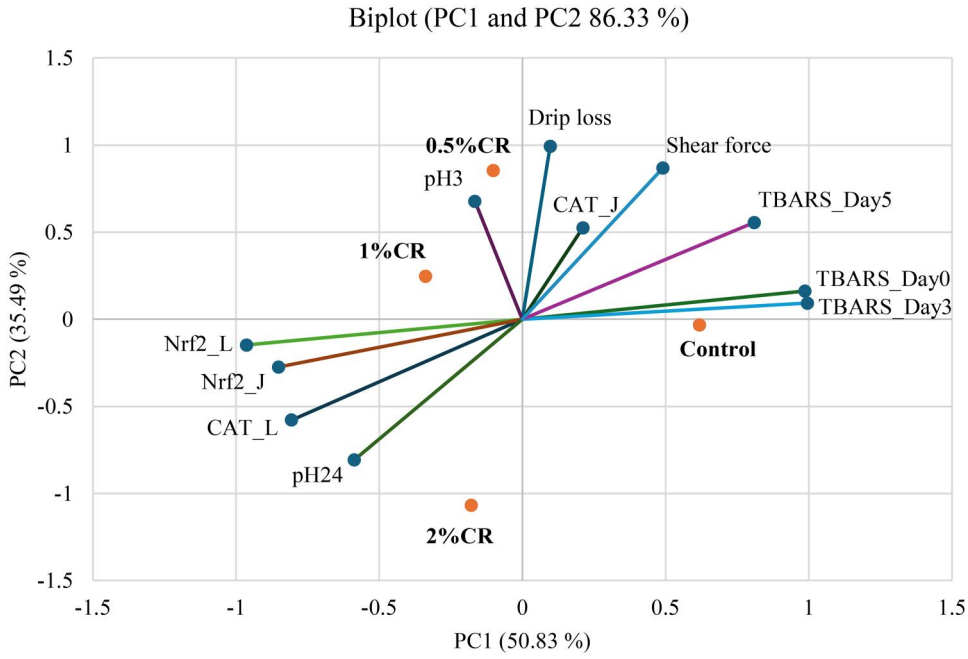

Figure 3. Principal component analysis biplot of meat quality and antioxidant related gene expression. CR = *Cannabis sativa* residue; CAT_J and CAT_L = catalase in jejunum and liver; Nrf2_J and Nrf2_L = nuclear factor erythroid-2–related factor 2 in jejunum and liver. Thiobarbituric acid reactive substances (TBARS).

due to the antioxidative properties of CR. In contrast, the 2% CR diet may promote breast and intestine growth, potentially due to improved digestion and nutrient absorption at higher supplementation levels, though this is accompanied by a reduction in EC, suggesting a possible trade-off in resource allocation. These findings highlight the potential for varying CR supplementation levels to influence specific carcase traits.

Overall, the carcase traits in the CR groups were similar to those in the control group. Bień et al. (2024) reported that adding 3% *C. sativa* extract (CSE) to broiler diets did not significantly affect breast yield, leg yield, or dressing percentage. Similarly, Sopian et al. (2024) observed no adverse effects on carcase traits with CR supplementation in broilers at 40 days old. Marzoni et al. (2014) also found no significant variation in carcase yield between chickens receiving a blend of natural antioxidants and those in the control group. In contrast, Banday et al. (2024) found that supplementing diets with 1.5% *Malva sylvestris* leaf powder significantly increased slaughter weight, dressed weight, and breast yield. The present study suggests that CR supplementation does not introduce toxic substances that impair carcase development, as evidenced by the lack of major differences across most traits.

In this experiment, there were no significant differences in the relative weight of visceral organs between the CR-supplemented and control groups. However, the 2% CR group showed the highest relative intestine weight among all the CR treatments. The higher relatively small intestine weight in the 2% CR group may be attributed to the bioactive compounds in CR, such as cannabinoids, terpenes, and flavonoids (Lewis et al. 2017; Fordjour et al. 2023), which may positively influence gut health. These compounds could stimulate the gut, enhancing intestinal activity, promoting cell proliferation, or inducing changes in gut morphology. The 2% CR group may have experienced more pronounced effects from these compounds, resulting in a higher relatively small intestine weight. Furthermore, our previous study (Sopian et al. 2024) found that CR supplementation improved the ratio between villus height and crypt depth in the ileum, suggesting beneficial effects on intestinal structure and function. In contrast, no significant variation was found in the intestine relative weight in older chickens supplemented with CR, although it affected heart relative weight (Sopian et al. 2024). These differences may be partly explained by the older age and longer supplementation period (Pascual et al. 2022). In line with our findings, Mashayekhi et al. (2018) reported that supplementation with medicinal herbs had no impact on the relative weights of the spleen and liver. Similarly, Bień et al. (2024) observed unaffected liver weight in non-stressed broilers fed CSE. However, in stressed chickens, liver weight was

reduced with 3% CSE supplementation, potentially promoting a healthier liver environment by alleviating oxidative stress. Because changes in liver weight are often associated with acute inflammatory responses (Mireles et al. 2005), indicating that CR supplementation did not negatively impact liver function. Moreover, the relative weights of other visceral organs remained unaffected by CR supplementation.

Regarding meat quality, the study revealed that the pH_{24} was significantly higher in the 2% CR group compared to both the control and 0.5% CR groups. In addition, supplementation with 2% CR improved meat tenderness and reduced drip loss, as evidenced by lower drip loss in both breast and thigh meats and reduced shear force in breast meat. These improvements in meat quality may be attributed to the bioactive compounds in CR, which could contribute to improve muscle integrity and water-holding capacity. Similarly, Bień et al. (2024) reported that supplementing broilers challenged with *Clostridium perfringens* with CSE reduced drip loss and increased breast meat pH. Other studies have also shown that feeding broilers phenolic-rich plants, such as bamboo leaf (Shen et al. 2019) and *Neolamarckia cadamba* leaf extract (Park et al. 2014), enhances meat quality, likely due to their antioxidant properties. Adeyemi (2021) demonstrated that natural antioxidants can protect myofibrillar proteins from oxidative degradation, as indicated by reduced carbonyl content in supplemented birds. However, some studies have reported no significant effects of medicinal plant extracts on meat quality traits. For instance, Park et al. (2014) found that a plant extract had no impact on breast muscle pH, drip loss, tenderness, or colour in broilers. These inconsistencies may result from differences in plant composition, bioactive compound concentrations, or experimental conditions. The findings of the present study suggest that the observed improvements in meat quality in the CR-supplemented groups are likely due to the combined effects of CR's bioactive compounds, particularly their antioxidant properties, which may help maintain muscle integrity and reduce protein oxidation.

The significant interaction between treatment and storage time observed in TBARS levels highlighted the effectiveness of CR in mitigating lipid oxidation in broiler meat. Lipid oxidation, as indicated by TBARS values, is a critical factor influencing meat quality during storage, with higher values reflecting oxidative deterioration and reduced shelf life (Adeyemi 2021). In the present study, CR supplementation significantly reduced TBARS levels in breast meat, regardless of inclusion level or storage duration, underscoring its potential as a natural antioxidant. The minimal differences in TBARS levels among CR-supplemented groups on days 0 and 3 suggested that even a low CR inclusion level (0.5%) provided sufficient antioxidative protection during the initial storage period. However, the superior performance of the 1% and 2% CR groups on day 5 emphasises the importance of higher supplementation levels in preserving meat quality during prolonged storage. These findings align with previous research demonstrating that the oxidative stability of broiler meat can be enhanced through the inclusion of medicinal plants rich in natural antioxidants, such as *Lonicera japonica* and *Ocimum basilicum* leaves (Park et al. 2014; Fathi et al. 2022). The results of the present study suggest that CR effectively delays lipid oxidation, likely due to its phytochemical content. Previous research has shown that *C. sativa* leaves possessed potent antioxidant properties, which were attributed to their phytocannabinoids, polyphenols, and flavonoids (Marsh and Smid 2020; Nakkliang et al. 2022). These bioactive compounds may protect against oxidative damage by scavenging free radicals or converting them into less reactive forms, thereby interrupting the chain reactions of lipid oxidation (Atalay et al. 2021). Consequently, CR supplementation could play a significant role in extending the shelf life of meat under refrigerated conditions.

Current research on the use of Cannabis as a feed additive in broiler diets remains limited. Among the most commonly studied by-products are hemp seed and hemp seed cake, typically used as alternative protein sources. He et al. (2025) demonstrated that hemp seed cake improved meat colour and reduced cooking loss in broilers. Similarly, Tufarelli et al. (2023) reported that hemp seed cake supplementation significantly lowered MDA and lipid hydroperoxide levels in breast meat, highlighting its antioxidative potential. In contrast, Serrapica et al. (2025) observed no significant effects on meat colour or proximate composition in organic broilers fed hemp seed. However, Tignani et al. (2024) found that hemp supplementation increased antioxidant compounds such as retinol and γ-tocopherol, supporting the potential health benefits observed in the present study. Beyond hemp products, other phytogenic additives, including cinnamon bark (Qaid et al. 2024) and green tea and mulberry leaves (Aziz-Aliabadi et al. 2025), have also been reported to improve meat quality by enhancing water-holding capacity, reducing shear force, and decreasing lipid oxidation. Collectively, these findings reinforce the hypothesis that plant-derived

bioactive compounds play an important role in improving oxidative stability and meat quality in broiler chickens.

In this study, chickens fed with CR exhibited enhanced antioxidant capacity, as evidenced by increased liver expression of Nrf2 and CAT genes, along with lower MDA levels in meat. Similar findings were reported by Sopian et al. (2024), who observed that dietary CR supplementation reduced plasma MDA content. The antioxidant effects of CR were likely attributed to its bioactive compounds, particularly CBD and THC, which were well-documented for their potent antioxidant properties (Nadeem et al. 2012; Dawidowicz et al. 2021). Consistently, Wang C et al. (2024) reported that supplementation with *N. cadamba* leaf extract upregulated the mRNA expression of key antioxidant markers including CAT, GSH-Px, and Nrf2 in broilers. Additionally, Xiao et al. (2024) suggested that the inclusion of natural antioxidants activates the Nrf2/antioxidant response element (ARE) pathway, thereby elevating CAT expression. The present study supports these findings, showing that dietary CR supplementation boosts the liver's antioxidant capacity in broilers *via* the Nrf2/ARE pathway, as indicated by increased Nrf2 and CAT expression. These results align with the study of İlhan et al. (2025), which demonstrated that CBD exerts significant antioxidant effects in rats by upregulating Nrf2 expression and alleviating apoptosis through the reduction of endoplasmic reticulum stress. Furthermore, CBD supplementation in pulmonary hypertension rat models has been shown to elevate antioxidant compound levels (Krzyżewska et al. 2022). Bioactive compounds present in CR, particularly polyphenols and cannabinoids, are known to modulate antioxidant defence mechanisms through several interconnected pathways. Polyphenols can activate the Nrf2 signalling cascade by promoting the dissociation of Nrf2 from its cytoplasmic inhibitor, Kelch-like ECH-associated protein 1 (Keap1), under conditions of oxidative or electrophilic stress (Tonelli et al. 2018). Once translocated to the nucleus, Nrf2 binds to ARE in the promoter regions of target genes, inducing the transcription of key antioxidant enzymes such as SOD, GPx, and CAT (Kaspar et al. 2009). Additionally, cannabinoids such as CBD possess direct antioxidant properties by scavenging reactive oxygen species and modulating redox-sensitive signalling pathways, including the inhibition of nuclear factor-kappa B (NF-κB) activation, which plays a central role in inflammatory and oxidative stress responses (Atalay et al. 2019).

Diets rich in antioxidants can potentially reduce pro-inflammatory responses and strengthen the immune system by modulating immune responses to pathogens and influencing cell signalling (Al-Khalaifah et al. 2024). However, in this study, no significant differences were observed among treatment groups in the expression of IFN-γ, IL-1ß, TNF-α, or IL-10 genes. This is consistent with the findings of Sopian et al. (2024), who also reported no significant effects of CR on immune-related organs in broilers. Teng et al. (2023) proposed that the expression of IL-1ß, IL-10, and IFN-γ is closely linked to parasite-induced stress. Likewise, Ünlü et al. (2024) suggested that the protective effects of *C. sativa* extract (99.9% CBD, $<$0.01% THC) may be attributed to its ability to inhibit the inflammatory process under conditions of oxidative stress. Although the present study focused on selected immune-related genes mainly linked to oxidative stress and inflammatory regulation, it is well recognised that immune responses are multifactorial and extend beyond gene expression. Cannabinoids and other bioactive compounds from *C. sativa* have been reported to influence immune function through multiple pathways, including modulation of cytokine networks, regulation of immunoglobulin production, and effects on leukocyte activity (Nichols and Kaplan 2020). Therefore, future studies incorporating additional immunological parameters such as cytokine profiles, circulating immunoglobulin levels, and leukocyte function assessments would provide a more comprehensive understanding of the immunomodulatory potential of CR in broiler chickens.

The PCA results show a strong association between CR supplementation, antioxidant markers and meat quality. PC1 shows an inverse relationship between TBARS and key antioxidant markers, including Nrf2 expression in liver and jejunum and CAT expression in liver. This suggests that higher antioxidant activity, as indicated by increased Nrf2 and CAT expression, is associated with lower lipid oxidation. CR supplementation was positively associated with antioxidant markers at all inclusion levels, highlighting its role in improving oxidative stability, while the control group had higher TBARS levels, indicating greater lipid oxidation. PC2 is associated with meat quality, with 2% CR supplementation positively related to pH_{24} and negatively related to shear force, pH_3 and drip loss, suggesting improved tenderness and water holding capacity. These results highlight the clear effects of CR supplementation, particularly at 2%, on improving antioxidant defence mechanisms and meat quality, while the control group had higher lipid oxidation and poorer meat

characteristics. A positive relationship between CR and Nrf2 and CAT expression suggests that CR increases antioxidant capacity, which is consistent with previous studies indicating that bioactive phytochemicals mitigate oxidative stress by activating the Nrf2 pathway (Surai et al. 2019). The reduction in TBARS levels underscores the antioxidant effects of cannabis-derived compounds, which are known to scavenge free radicals and prevent lipid peroxidation (Dawidowicz et al. 2021; Muscarà et al. 2021). In addition, the improved meat quality characteristics in the CR supplemented groups, such as greater pH stability and lower drip loss, could be due to reduced oxidative damage, as oxidative stress is closely associated with meat quality deterioration (Adeyemi 2021; Banday et al. 2024). These results emphasise the dual role of CR in increasing antioxidant activity and improving meat quality in poultry production.

Limitations

Although the inclusion of CR resulted in slight variations in the chemical composition of the experimental diets, proximate analysis confirmed no significant differences in the major nutritional components among treatments. Therefore, the observed improvements in meat quality and antioxidant capacity can be largely attributed to the CR inclusion itself. Nonetheless, we acknowledge that formulating strictly iso-nutritional diets would allow for even clearer isolation of the specific effects of CR. Future research should aim to adjust basal diet formulations to fully control for nutrient levels across treatments.

Conclusion

This study shows that CR supplementation improves antioxidant capacity and meat quality in broiler chickens. While 1% CR improved eviscerated carcase yield, 2% CR increased breast and intestinal weight percentage, increased breast meat pH, and decreased shear force and drip loss, indicating better texture and water holding capacity. CR also lowered malondialdehyde levels in stored meat, which improved oxidative stability, and upregulated Nrf2 and CAT expression in liver and jejunum. Principal component analysis confirmed these effects and supported CR (0.5%–2%) as a potential feed additive to improve meat quality and oxidative stability in broilers.

From a practical standpoint, these findings suggest that CR could offer poultry producers a novel phytogenic additive to enhance meat quality and shelf life. However, the economic feasibility of using CR at scale and compliance with national and international regulatory standards must be carefully evaluated, particularly given the varying legal status of CR across regions. Future research should also address long-term safety, consumer acceptance, and cost–benefit analyses to support the responsible use of CR in commercial poultry production.

Acknowledgments

We gratefully acknowledge the School of Industrial Education and Technology, KMITL, and the Faculty of Agriculture, Chiang Mai University, for providing the research facilities. During the preparation of this work, the authors used ChatGPT 3.5 to improve readability and refine the clarity of the text. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Ethical approval

This experiment was approved by the ethical committee of the Faculty of Agriculture, Chiang Mai University (Approval No. AGIACUC015/2665).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Science, Research, and Innovation Fund through King Mongkut's Institute of Technology Ladkrabang (KMITL) under Grant [number RE-KRIS/FF67/010] and KMITL Doctoral Scholarship [KDS 2022/028].

Data availability statement

Data will be available upon reasonable request from the corresponding author.

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