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Meta-analysis of dietary supplementation with hemp products (*Cannabis sativa* L.) in broiler chicken: performances, blood profiles, intestinal morphology, and meat physicochemical parameters

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Abstract The results showed that dietary hemp products reduced plasma triglycerides (SMD = -2.263; P < 0.01), cholesterol (SMD = -1.239; P < 0.05), and low-density lipoprotein (SMD = -5.711; P < 0.01), whereas the plasma high-density lipoprotein (SMD = 3.193; P < 0.01) and jejunal villus height (SMD = 4.536; P < 0.01) were increased. However, broiler performances (average daily feed intake, body weight, feed conversion ratio) and meat physicochemical parameters (pH, lightness, redness, yellowness, crude protein, and ether extract) were not significantly affected. Therefore, the study indicated that dietary hemp products in broiler chicken could improve blood profiles and gut health, suggesting their potential as an alternative feedstuff in broiler production.

Keywords: Cholesterol, High-density lipoproteins, Low-density lipoproteins, Villus height

Introduction

Cannabis sativa L. is one of the most versatile plants globally. It has been grown for its fiber, seed, and medicinal properties (Xu *et al.,* 2021). The clasificaion of cannabis is primarily determined by its tetrahydrocannabinol (THC) content, which influences its legal classification. In the United States, cannabis must have a THC level below 0.3% to be categorized as Hemp, while in European countries, the threshold is set at 0.2% (Small, 2015). Currently, the growing global interest in cannabis has coincided with its legalization in many countries (EFSA, 2011; Small, 2015). Therefore, several studies have evaluated

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a range of hemp products nutrient content and its potential application, including hemp seeds (HS), hemp cake (HC), and hemp oil (HO). Vonapartis *et al*. (2015) and Karlsson *et al*. (2010) reported that whole HS contains 25.6% crude protein and 29.2% crude fat, whereas the HC reported higher crude protein content (34.4%) and lower fat (12.4%). Meanwhile, HO has a favorable fatty acid composition (Jing *et al*., 2017; Kanbur, 2022).

Recently, Hemp products have been studied as potential feedstuff in broiler chicken (Kanbur, 2022; Mahmoudi *et al*., 2015; Vispute *et al*., 2019). Khan *et al*. (2010) evaluated the effect of HS (5%, 10%, or 20%) in broiler chicken during 42 days of the experiment. The maximum of HS up to 20% increases body weight and feed efficiency. In addition, HC was fed to the broiler, resulting in no significant differences in body weight, feed intake, or feed efficiency (Eriksson and Wall, 2012). However, feeding HO harms those variables in young broiler chicks but improves the fatty acid profile of abdominal fat (Kanbur, 2022). Jing *et al.* (2017) also noticed that the HO supplementation increased favorable fatty acid content in broiler chickens' breast and thigh meat. Furthermore, Stastnik *et al*. (2019) investigated the impact of incorporating HC into broiler diets on the carcass characteristics of broiler chickens. Their findings indicated that adding HC at 5% and 15% levels did not significantly alter carcass yield, breast meat percentage, or thigh meat percentage. No notable differences were observed between broilers fed HC and those given HS or HO (Kanbur, 2022; Khan *et al*., 2009; Vispute *et al.,* 2019). Moreover, another study reported that HC inclusion in diets at 10% and 20% decreased abdominal fat (Eriksson and Wall, 2012). The differences in the results of several studies may be attributed to differences in the hemp products, broiler breed, dosage, and other factors.

Meta-analysis is an advanced statistical method used to quantitatively combine findings from all available and relevant studies on a particular research topic. This technique helps to identify the factors that affect the direction and strength of the relationships between variables (Gurevitch *et al.,* 2018). This statistical approach has recently been increasingly applied in broiler nutrition (Ogbuewu *et al.,* 2020; Ogbuewu *et al.,* 2023; Sopian *et al.,* 2024). Therefore, the current meta-analysis aimed to investigate the effects of hemp products supplementation on broiler performances, blood profiles, intestinal morphology, and meat physicochemical parameters from selected studies.

Materials and methods

The list of studies examining the use of hemp products in broiler chicken diets was compiled from PubMed, Google Scholar, and Scopus using search terms such as "hemp," "seed," "oil," "cake," and "broiler," combined with Boolean operators (AND/OR). Additionally, the reference lists of pertinent articles were manually checked to identify further relevant studies. The meta-analysis was included the controlled experiments evaluating the effects of hemp products on broiler chickens, and provided at least one relevant outcome with data for both control and experimental groups, mean values, the number of birds per dietary group, and measures of variability presented in numerical or graphical form. The selection process is illustrated in Figure 1.

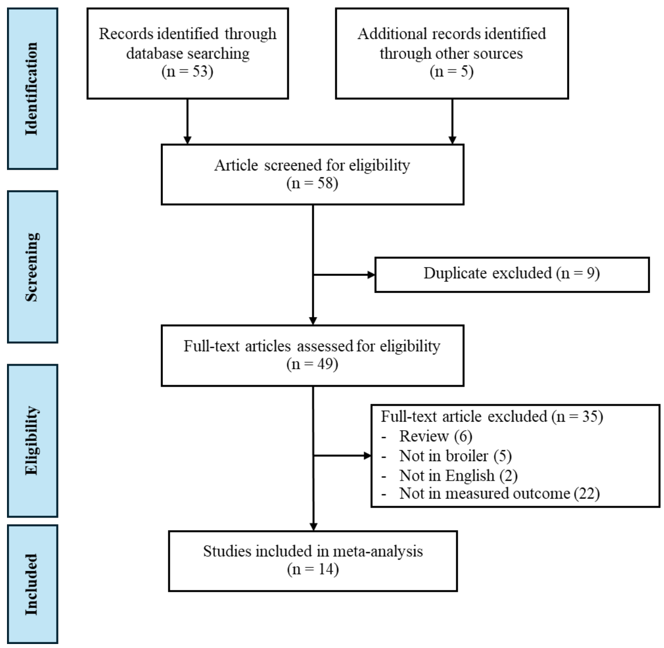

Figure 1. Article selection flow chart for the meta-analysis.

The meta-analysis was conducted using a random-effects model with the Open Meta-analyst for Ecology and Evolution (OpenMEE) software (Wallace *et al.,* 2017), utilizing the DerSimonian-Laird approach (DerSimonian and Laird, 2015). Results were reported according to Koricheva *et al.* (2013), as standardized mean differences (SMD) with a 95% confidence interval (CI). Pooled results were deemed significant at a 5% probability level. Heterogeneity was evaluated using the I^2 statistic (Higgins *et al.,* 2003). To identify the sources of heterogeneity, a subgroup analysis was conducted to examine the effects of moderator variables, specifically hemp products and inclusion levels. However, this was not done for subgroups with fewer than three studies due to low statistical power (Ogbuewu *et al.,* 2020). Publication bias was not assessed as the number of studies per outcome was less than ten (Ogbuewu *et al.,* 2023).

Results

A systematic search was done across three databases which identified 58 prospective studies, with 14 of these meeting the inclusion criteria. The selected studies for the analysis were conducted in eight countries spanning three continents. The earliest study had done from 2010, while the most recent was gathered from 2023. Hemp products' level of inclusion ranged from 0-30% HS, 0-24% HC, and 0-9% HO. The detailed characteristics of the studies included in this meta-analysis are described in Table 1.

The forest plot revealed that the broiler's performance, average daily feed intake (ADFI), was not significantly affected by hemp products (SMD = 0.057; 95% CI -0.208, 0.322; I = 31.93%; P > 0.05; Figure 2). Similarly, body weight (BW) (SMD = -0.626; 95% CI -1.721, 0.468; I = 93.73%; P > 0.05; Figure 3) and feed conversion ratio (FCR) (SMD = 0.207; 95% CI -0.079, 0.494; I = 65.16%; P > 0.05; Figure 4) had similar results as compared to control groups. Subgroup analysis by the level of inclusion and hemp products were illustrated in Table 2. The results showed that the level of inclusion of hemp products at ≤ 10% and >10% had similar performance compared to the control. However, broilers offered HO had lower FCR (SMD = -0.586; 95% CI -1.042, -0.131; P < 0.05) and BW (SMD = -6.221; 95% CI -8.695, -3.747; P < 0.01) than the control, whereas HS had higher FCR than the control (SMD = 0.618; 95% CI 0.228, 1.007; P < 0.05). No significant differences were found in HC compared to the control (P > 0.05).

The broiler blood profiles fed with hemp products is shown in Table 3. There were significantly lower triglycerides (SMD = -2.263; 95% CI -3.265, -1.261; P < 0.01), cholesterol (SMD = -1.239; 95% CI -2.286, -0.192; P < 0.05), and low-density lipoproteins (LDL) (SMD = -5.711; 95% CI -8.843, -2.579; P < 0.01), but higher high-density lipoproteins (HDL) (SMD = 3.193; 95% CI 1.08, 5.307; P < 0.01) in broiler chickens fed hemp products than controls. However, the treatment diets did not affect aspartate transaminase (AST) and alanine transaminase (ALT) (P > 0.05).

The influence of hemp products on intestinal morphology and meat physicochemical parameters in broiler chickens is shown in Table 4. The hemp products increased villus height (VH) (SMD = 4.536; 95% CI 2.259, 6.813; P < 0.01) in broiler chicken. However, the treatments had no significant effect on crypt depth (CD) and VH/CD compared with the control. Similarly, the treatment groups had comparable content of crude protein (CP), ether extract (EE), pH, and meat color (lightness (L*), redness (a*), and yellowness (b*)) of chicken meat with the control groups.

Table 1. Overview of studies included in the meta-analysis

Authors	Country	Continent	Inclusion (%)	Hemp products	Outcomes
Jing *et al.* (2017)	Canada	North America	0, 3, 6	Hemp Oil	ADFI, FCR
Eriksson and Wall (2012)	Sweden	Europe	0, 10, 20	Hemp Cake	BW, FCR
Taubner *et al.* (2023)	Czech	Europe	0, 4	Hemp Seed	BW, FCR
Vispute *et al*. (2019)	India	Asia	0, 0.2, 0.3	Hemp Seed	BW, FCR, TG, TC, HDL, LDL, ALT, AST, VH, CD, VH/CD
Skrivan *et al*. (2020)	Czech	Europe	0, 4	Hemp Seed	BW, FCR
Mahmoudi *et al.* (2015)	Iran	Asia	0, 2.5, 5, 7.5	Hemp Seed	ADFI, ADG, FCR, TG, TC, HDL, LDL, ALT, AST
Sana *et al.* (2024)	Pakistan	Asia	0, 10, 15, 20	Hemp Seed	FCR, VH, CD, VH/CD
Khan *et al.* (2010)	Pakistan	Asia	0, 5, 10, 20	Hemp Seed	BW, FCR
Stastnik *et al.* (2019)	Czech	Europe	0, 5, 15	Hemp Cake	BW, CP, EE, pH, L*, a*, b*
Tufarelli *et al.* (2023)	Italy	Europe	0, 5, 10	Hemp Cake	BW, ADFI, FCR, CP, EE, pH, L*, a*, b*
Rasool (2018)	Canada	North America	0, 10, 20, 30; 8, 16, 24; 3, 6, 9; 0, 6, 12, 18; 5, 10, 15; 2, 4, 6	Hemp Seed, Hemp Cake, Hemp Oil	ADFI, FCR
Kanbur (2022)	Turkey	Asia	0, 1.22, 3.05, 6.10	Hemp Oil	BW, FCR, pH, L*, a*, b*, TG, TC, HDL, LDL, ALT, AST
Vispute *et al*. (2021)	India	Asia	0, 0.2, 0.3	Hemp Seed	pH
Stastnik *et al.* (2016)	Czech	Europe	0, 2.5	Hemp Cake	BW

[1]BW, body weight; ADFI, average daily feed intake; FCR, feed conversion rate; TC, total cholesterol; TG, trygliceride; ALT, alanine transaminase; AST, aspartate transaminase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CD, crypth depth; VH, villus height; VH/CD, villus height to crypth dept ratio; CP, crude protein; EE, ether extract; L*, lightness; a*, redness; b*, yellowness.

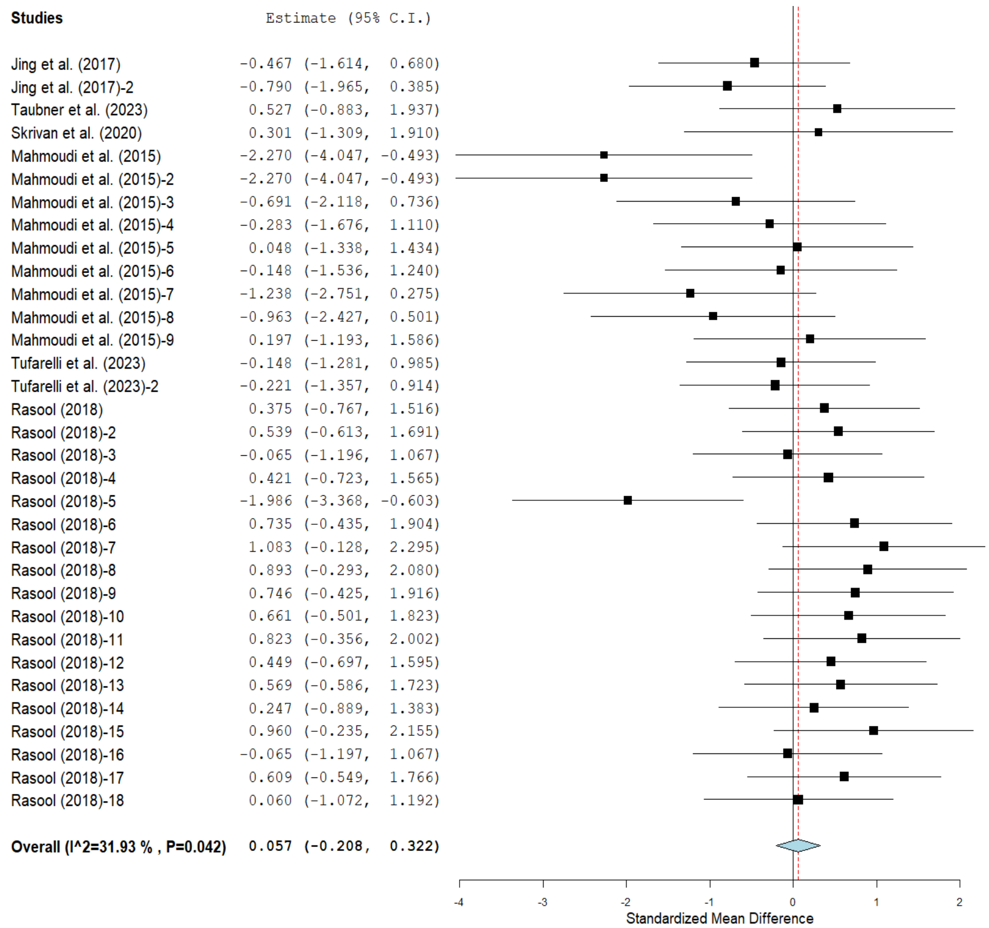

Figure 2. Forest plot of the standardized mean difference (SMD) and 95% confidence interval of Hemp products on broiler average daily feed intake (ADFI), Pooled estimates are regarded as significant when the solid line does not intersect the diamond at the bottom of the forest plot (Koricheva *et al.,* 2013)

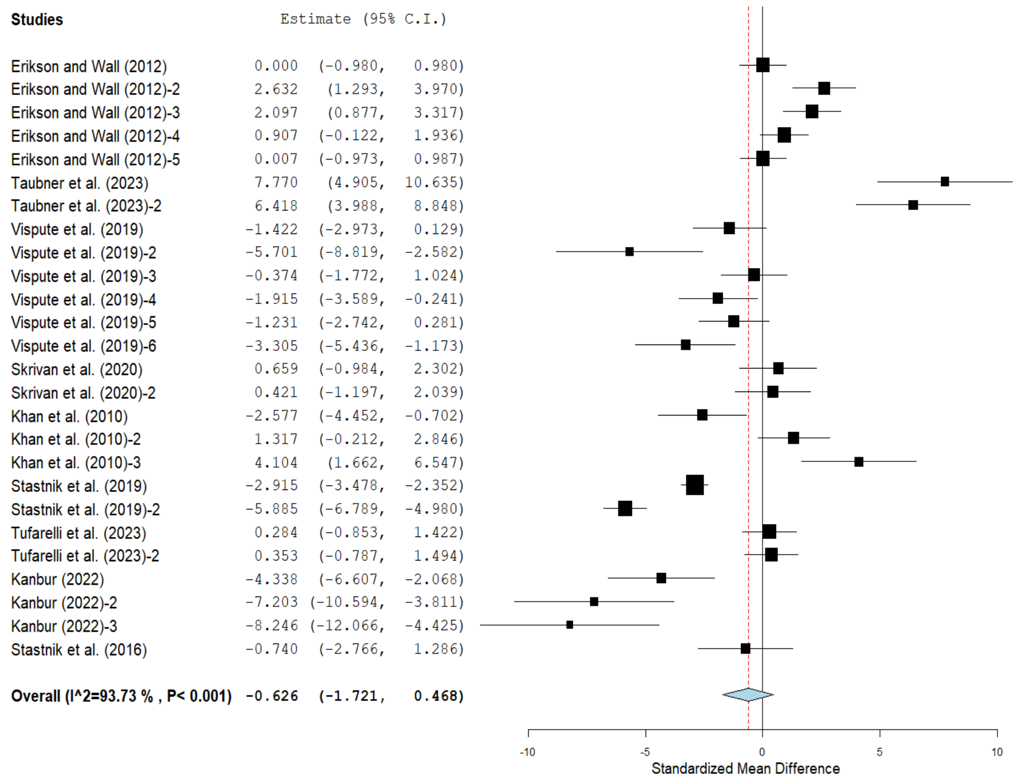

Figure 3. Forest plot of the standardized mean difference (SMD) and 95% confidence interval of Hemp products on broiler body weight (BW), Pooled estimates are regarded as significant when the solid line does not intersect the diamond at the bottom of the forest plot (Koricheva *et al.,* 2013)

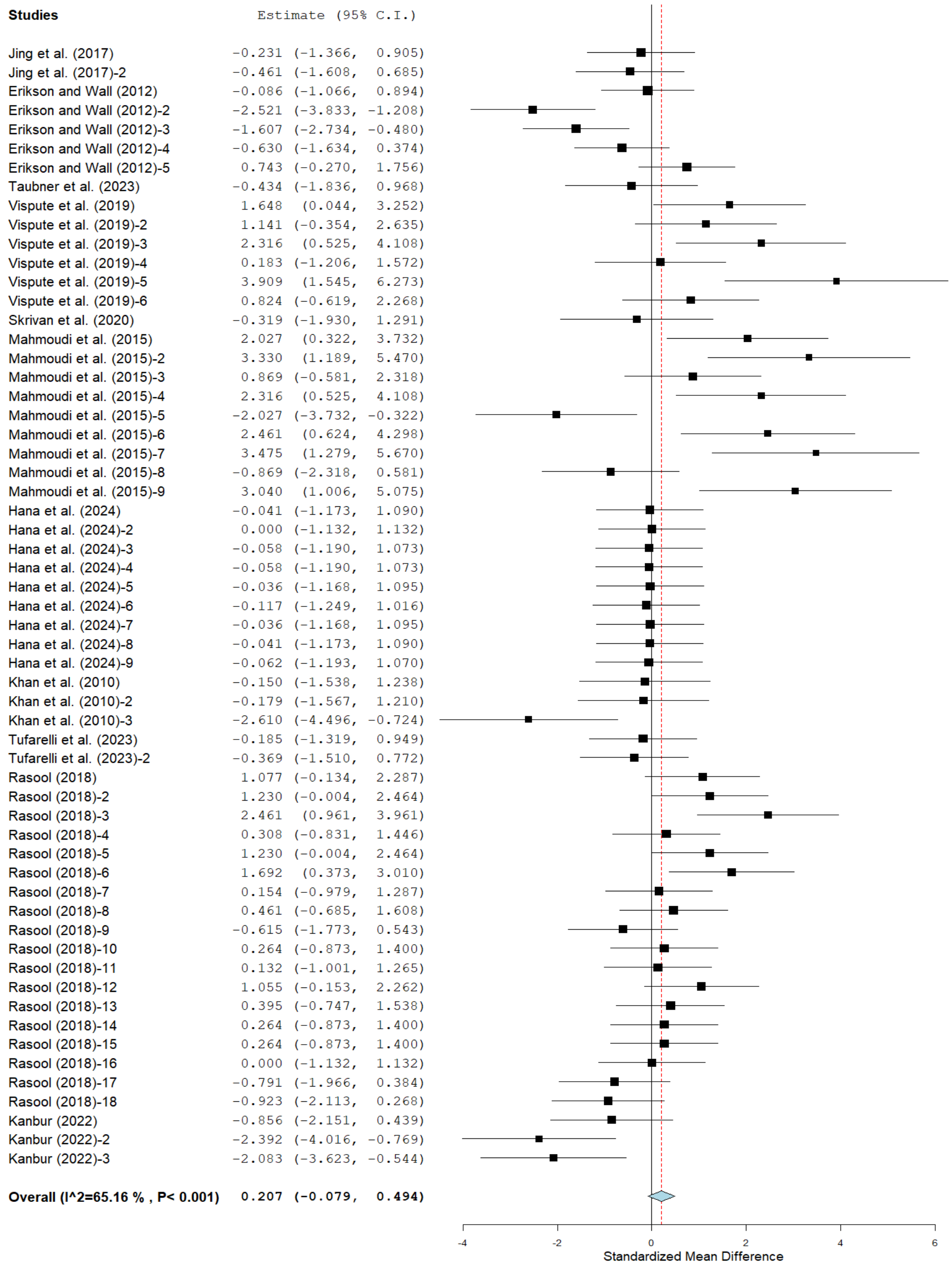

Figure 4. Forest plot of the standardized mean difference (SMD) and 95% confidence interval of Hemp products on broiler feed conversion ratio (FCR), Pooled estimates are regarded as significant when the solid line does not intersect the diamond at the bottom of the forest plot (Koricheva *et al.,* 2013)

Table 2. Subgroup-analysis of the effect of level of inclusion and hemp products (*Cannabis sativa* L) on broiler performances[1]

Variables[2]	Covariate	Subgroup[3]	SMD	95% CI		SE	P-value
				Lower	Upper		
ADFI	Inclusion (%)	≤ 10	0.003	-0.281	0.286	0.145	0.986
		> 10	0.252	-0.424	0.929	0.345	0.465
	Hemp products	HS	-0.097	-0.494	0.300	0.202	0.632
		HC	0.115	-0.459	0.689	0.293	0.695
		HO	0.247	-0.218	0.711	0.237	0.298
BW	Inclusion (%)	≤ 10	-0.792	-1.905	0.321	0.568	0.163
		> 10	0.174	-3.181	3.529	1.712	0.919
	Hemp products	HS	0.262	-1.287	1.811	0.790	0.740
		HC	-0.347	-2.005	1.311	0.846	0.682
		HO	-6.221	-8.695	-3.747	1.262	< 0.001
FCR	Inclusion (%)	≤ 10	0.206	-0.153	0.566	0.183	0.261
		> 10	0.230	-0.249	0.708	0.244	0.347
	Hemp products	HS	0.618	0.228	1.007	0.199	0.002
		HC	-0.039	-0.593	0.514	0.282	0.890
		HO	-0.586	-1.042	-0.131	0.232	0.012

[1]SMD, standardized mean differences; CI, confidence interval; SE, standard error.
[2]ADFI, average daily feed intake; BW, body weight; FCR, feed conversion rate.
[3]HS, hemp seed; HC, hemp cake; HO, hemp oil.

Table 3. Blood profiles of broilers fed hemp products[1]

Outcomes[2]	N	SMD	95% CI		SE	P-value	Heterogeneity	
			Lower	Upper			I^2	P-value
Triglycerides	8	-2.263	-3.265	-1.261	0.511	< 0.001	83.30	< 0.001
Cholesterol	8	-1.239	-2.286	-0.192	0.534	0.020	87.86	< 0.001
HDL	5	3.193	1.080	5.307	1.078	0.003	88.98	< 0.001
LDL	5	-5.711	-8.843	-2.579	1.598	< 0.001	91.02	< 0.001
AST	5	-0.817	-2.767	1.133	0.995	0.412	90.96	< 0.001
ALT	5	0.787	-0.185	1.758	0.496	0.113	81.09	< 0.001

[1]N, number of comparisons; SMD, standardized mean differences; CI, confidence interval; SE, standard error, I^2, inconsistency index.
[2]LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALT, alanine transaminase; AST, aspartate transaminase.

Table 4. Intestinal morphology and meat physicochemical parameters of broilers fed hemp products[1]

Outcomes[1]	N	SMD[2]	95% CI		SE	P-value	Heterogeneity	
			Lower	Upper			I^2	P-value
Jejunal histomorphology								
VH	5	4.536	2.259	6.813	1.162	< 0.001	83.65	< 0.001
CD	5	0.464	-0.051	0.979	0.263	0.077	0.00	0.909
VH/CD	5	0.479	-0.038	0.995	0.263	0.069	0.00	0.839
Meat physicochemical parameters								
CP	4	0.111	-0.484	0.705	0.304	0.716	0.00	0.795
EE	4	-0.807	-2.510	0.896	0.869	0.353	83.72	< 0.001
pH	9	0.124	-0.264	0.513	0.198	0.531	0.00	0.983
L*	7	0.234	-0.080	0.549	0.161	0.145	0.00	0.73
a*	7	-0.072	-0.385	0.242	0.160	0.654	0.00	0.806
b*	7	-0.617	-1.513	0.278	0.457	0.176	84.92	< 0.001

[1]N, number of comparisons; SMD, standardized mean differences; CI, confidence interval; SE, standard error, I^2, inconsistency index.
[2]VH, villus height; CD, crypth depth; VH/CD, villus height to crypth dept ratio; CP, crude protein; EE, ether extract; L*, lightness; a*, redness; b*, yellowness.

Discussion

The pooled results indicated that dietary hemp products did not negatively affect broiler performance (ADFI, BW, and FCR). These findings are consistent with previous research by Sana *et al.* (2024), Tufarelli *et al.* (2023), and Jing *et al.* (2017), all of which reported no significant impact of hemp products on broiler performance. However, subgroup analyses revealed that supplementation with HO led to lower body weight than the control group. This observation aligns with Kanbur (2022), who also found a reduction in BW among young broilers receiving HO. In contrast, no significant differences in BW and ADFI were observed for the HS and HC treatments. This result agrees with Rasool (2018), who found no effect of hemp products (HS, HO, and HC) on growth performance in broiler diets. In addition, Jing *et al.* (2017) found no discernible effect of HO supplementation at 3% and 6% levels in broilers during the starter period (1-21 days). However, Khan *et al.* (2010) reported improved performance with a 20% HS inclusion in the diet compared to the control. This could be attributed to the HS richness in fatty acids content, high-quality amino acids and other essential components, including vitamins and minerals. Overall, these results support the potential use of hemp products in broiler feed. Nevertheless, it is important to consider that the effects of dietary hemp products may vary depending on factors such as form, dosage, duration, broiler strain, and other experimental conditions (Sana *et al.,* 2024).

Blood parameters are frequently used in nutritional research to evaluate the impact of unconventional feed ingredients (Ogbuewu *et al.,* 2015). The results from recent studies indicate that hemp products influence the blood biochemical profile of broiler chickens by reducing plasma triglycerides, cholesterol, and low-density lipoprotein (LDL) levels while simultaneously increasing high-density lipoprotein (HDL) levels. These modifications in serum lipid profiles are attributed to the distinctive lipid composition of hemp seeds, which are abundant in essential fatty acids (Vispute *et al.,* 2019). Additionally, the supplementation of polyunsaturated fatty acids (PUFAs) in poultry diets has been shown to lower blood cholesterol and overall lipid levels (Alagawany *et al.,* 2019). Hemp seeds are particularly rich in essential fatty acids, including omega-6 and omega-3, which make up to 90% of their total fatty acid content (Callaway, 2004). The study also found no significant differences in alanine transaminase (ALT) and aspartate transaminase (AST) levels across the dietary groups. ALT levels typically increase with liver cell injury and are notably elevated in cases of toxin-induced liver damage, whereas AST levels rise with tissue necrosis and chronic liver conditions (Gowda *et al.,* 2009). Therefore, the study suggests that dietary hemp products did not alter broiler liver function.

Understanding diet's effects on the intestinal tract's morphological characteristics is essential for evaluating digestive efficiency. This efficiency depends on the enteric mucosal surface and the functional properties of the epithelial cells (Stillhart *et al.,* 2020). In the present meta-analysis, hemp products enhanced intestinal morphology by increasing villus height (VH) in broiler chickens. However, no significant differences were observed in crypt depth (CD) or the VH/CD ratio. Similarly, Tufarelli *et al.* (2023) reported increased VH and VH/CD ratios in broilers fed with 5% and 10% HC in the diets. In contrast, no change has been observed in the intestinal morphology of the broilers with dietary HS up to 20% inclusion (Sana *et al.,* 2024). The height of the intestinal villi is directly linked to the rate at which nutrients are absorbed and overall performance improvements (Vispute *et al.,* 2019). Recent research by Taubner *et al.* (2023) has highlighted that HS contains biologically active compounds that may influence digestion by interacting with various receptors and enzymes. Additionally, dietary supplementation with HS has been shown to enhance gut microbiota homeostasis (Opyd *et al.,* 2020), thus preventing dysbiosis and strengthening the mucosal barrier against pathogenic bacteria (Rubio, 2019).

The color of chicken meat could influence consumer preferences, and the degree of skin pigmentation is influenced by the presence of lipid-soluble pigments like carotenoids in the feed (Mir *et al.,* 2017). The meta-analysis indicated that dietary hemp products did not affect CP, EE, pH, or meat color. Similarly, Tufarelli *et al.* (2023) reported that dietary HC did not affect meat physicochemical parameters. In addition, HO and HS supplementation in the broiler diet did not affect meat pH, but a high level of inclusion was reported to affect the yellowness of meat color (Kanbur, 2022; Stastnik *et al.,* 2019).

In conclusion, the results of this meta-analysis demonstrated that adding hemp products to broiler diets had no adverse effect on performance, blood biochemistry profile, intestine morphology, or meat physicochemical parameters. Moreover, dietary hemp products improved lipid plasma profile and villus height. Therefore, hemp products could be recommended as an alternative feedstuff in broiler production.

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