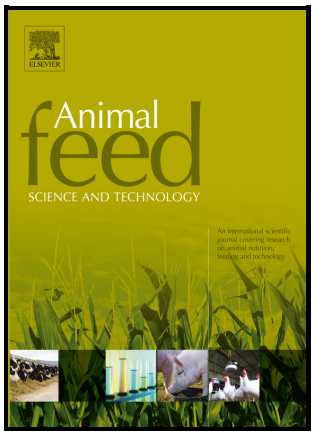
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Animal Feed Science and Technology

Hemp (*Cannabis sativa* L.) flower residue from cannabidiol extraction in laying hens: Feeding value and cannabinoid transfer to, and washout from plasma and muscle

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A B S T R A C T

The residue of cannabidiol (CBD) extraction from flowers of high-CBD-low-tetrahydrocannabinol (THC) hemp is a novel industrial byproduct but still prohibited for use as

feed in food-producing animals. Feeding value and cannabinoid transfer into body tissues was tested in a 5-week experiment with 3×15 laying hens allocated to a Control, Alfalfa or Hemp group. The Alfalfa group received the control diet diluted with 150 g/kg alfalfa. After an adaptation period gradually replacing alfalfa with hemp residue, the Hemp group received 150 g/kg hemp residue in the control diet. Five additional hens were first hemp-fed then received alfalfa during 9 days before slaughter (Washout group). Feed intake and eggs laid were recorded. Samples of feeds, eggs, excreta, blood plasma and breast muscle were collected. Feeds, plasma and muscle of the hens were analyzed for cannabinoids with adapted methods. Performance of hens fed hemp matched that of the Alfalfa and even of the Control group despite less metabolizable energy intake. Egg yolk color was most intense with hemp. The hemp flower residue contained 122 and 6 mg/kg of total CBD and THC. The plasma of Hemp hens contained 3,393 and 402 µg/L of total CBD and THC while respective levels in muscle were 139 and 47 µg/kg which exceeded the range reported for meat of hempseed-fed ruminants. Muscle concentrations of total CBD and THC of the Washout group were as low as 8.2 and 0.2 µg/kg, respectively. Overall, hemp flower residue showed potential in poultry nutrition.

Keywords: Hemp, Cannabinoid, Alfalfa, Poultry, Nutrition

1. Introduction

Due to the changing legal context in many countries, there is an increase in the cultivation of hemp cultivars containing less than 3 g/kg Δ^9-tetrahydrocannabinol (THC) on a dry matter (DM) basis [e.g., European Union, 2021]. This especially includes an increased production of hemp (*Cannabis sativa* L.) flowers for cannabidiol (CBD) extraction to address the global demand for CBD for use in wellness products ranging from food and beverages to cosmetics (Li et al., 2021). Unpollinated female hemp flowers are an abundant source of CBD and other cannabinoids compared to other parts of the plant (Kleinhenz et al., 2020a). The hemp cultivars for CBD production, selected for high flower yield and cannabinoid content, are distinct in

morphology from fiber type cultivars. These cultivars are highly branched, short, and often have low fiber density. In contrast, fiber hemp varieties often possess a single unbranched main stem, are very tall, have minimal flowers and a high fiber content. Some producers intensify yield by cultivation in greenhouses where two or more harvests per year are possible. After extraction of the target cannabinoids, including CBD and numerous other cannabinoids including THC, the hemp flower residue [also called 'extracted flower' by Kleinhenz et al. (2020a) and 'spent hemp biomass' by Irawan et al. (2025a)], often ends up as compost material due to the current lack of other valorization channels. The flower residues from cannabinoid extraction were analyzed to have a high crude protein content (250 g/kg DM) compared to other hemp plant parts (even more than the unextracted flowers; Kleinhenz et al., 2020a) highlighting its potential as poultry feed. In addition, its pigments, not lost during extraction, may assist in natural egg yolk coloration like the common grass or alfalfa meal additions.

The possibility of hemp cannabinoid transfer from animal feed to food and the large variation in cannabinoid abundance in different hemp plant parts motivated policymakers to distinguish which parts are suitable as feed for food-producing animals and which are not (reviewed by Taşkesen and Tüfekci, 2025). However, there are substantial differences among countries in the respective regulations. In the United States, only hempseed meal has been approved as a protein supplement for laying hens (AAFCO, 2024; Fruge et al., 2025). No hemp-based material is considered as a feed in Canada (Neijat and House, 2024). By contrast, Australia and New Zealand allow hempseed, leaves and flowering heads, and specify max. concentration limits for both THC and CBD (Taşkesen and Tüfekci, 2025). The feed catalogue of the European Union (2013), relevant also for laying hens includes hempseed, expeller and oil, because hempseed is considered by the EFSA (2011) to be 'practically free of THC' with max. 12 mg THC/kg. The European Union further permits flour (ground dried leaves) and fiber (product obtained during the processing of hemp; green colored, dried, fibrous) to be fed. The Swiss regulations (EAER, 2023) allow feeding of the same materials as the European Union,

except hemp leaves, stems (meal and fiber), and extracts (such as CBD). Overall, owing due to their very high THC concentrations, hemp flower material is rarely included in the lists of approved feed materials.

Hemp flower residue may be a novel, previously unconsidered, feed candidate because the industrial processing aims at most efficient extraction of cannabinoids. This likely results in substantially lower cannabinoid concentrations in the residue compared to the unprocessed hemp flower. However, as no legal distinction is currently made, hemp flower residue is not approved for feeding in many countries. Furthermore, studies about the actual concentration of such residues and, generally, the transfer of cannabinoids from hemp-based feeds to poultry-sourced foods are lacking. The few studies investigating and recovering cannabinoid residues in body tissues and blood plasma in farm animals were carried out with ruminants. These were calves offered industrial hemp (Kleinhenz et al., 2020b), sheep fed hemp stubble (Krebs et al., 2021) or hemp hay (Stevens et al., 2022), beef heifers fed hempseed cake (Smith et al., 2023), sheep, dairy cows and bees steers fed spent hemp biomass (Irawan et al., 2024, 2025b, 2026), and beef steers fed hemp leaves (Fruge et al., 2025). Some studies also investigated the reduction of cannabinoid concentrations after terminating the use of hemp-based feeds. Fruge et al. (2025) found a clearance of plasma after 4 days had passed, whereas levels in adipose tissue were persistent for at least 6 weeks. After about 4 weeks of washout, THC was still detectable in adipose tissue of sheep and cattle, but no longer in other body tissues (Irawan et al., 2024, 2025b, 2026). To our knowledge, none of the studies tested the residues resulting from the cannabinoid extracted hemp flowers in poultry. Potential species-specific differences in absorption and tissue distribution may limit transferability of findings in ruminants. In addition, no peer-reviewed study has reported results on the feeding value of hemp flower residue – beyond describing its nutrient composition – for non-ruminants in general and for laying hens in particular.

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The objective of this study was, therefore, to assess the feeding value and level of accumulation of CBD and THC when feeding CBD-hemp flower residue to laying hens. In detail, the following hypotheses were tested: a) The feeding value of hemp flower residue is at least as high as that of alfalfa meal; b) CBD and THC can be detected in blood, being the entry point from the gut, but not or only in low concentrations in the meat and in limited concentrations in the egg; c) 9 days of washout, achieved by withdrawal of the hemp flower residue from the diet, are sufficient to substantially reduce CBD and THC in blood and muscle tissue.

2. Material and methods

2.1. Animals

All procedures involving the hens were covered by the authorization from the cantonal veterinary office of Zurich, Switzerland (license no. 221/17). Fifty 72-week old white-laying Super Nick (H&N International) hens which were approximatively 45 weeks in lay were purchased from a commercial farm and housed at AgroVet-Strickhof, Lindau, Switzerland. Hens at this late laying stage were chosen as the assumed reduced feeding value of diets including alfalfa or hemp flower residue would be still sufficient to cover their requirements. Housing and experimental techniques were as applied and described earlier (Heuel et al., 2021). Briefly, the hens were kept individually in enriched, 80 × 80 × 80 cm sized cages equipped with a feeding trough, two nipple drinkers, sand bath, sitting pole and nest. This construction allowed measuring individual feed intake and the complete collection of eggs and excreta (on a tray after removing the sand bath). Feed and water were available at *ad libitum* access. Temperature was maintained at 20°C and 40 Lux were provided from 5.00 h to 21.00 h.

2.2. Feeds and diets

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Three experimental feeds were used. The first was a basal feed, which was part of all experimental diets. This feed was represented by a commercial mash compound feed designed for hens in lay for 45 weeks or longer (S-8860, Phase 3 NPr FreeFlow® Amrein, Neuenkirch, Switzerland). Alfalfa was the second control feed. It was purchased in pelleted form from Original Futtermühle (Felix Bühler, Sihlbrugg, Switzerland) and was ground to pass a 0.5 mm sieve before use in the experiment. The third feed consisting of the dried residue from hemp flower CBD extraction. Untrimmed flowers (with a small leaf proportion) had been obtained from the winter harvest of the hemp cultivar Pure Red 2 (developed and produced by Puregene AG), a cultivar selected for its CBD abundance in flower and compliant THC content. Concentrations measured (method as described in Chapter 2.4.4.) in different batches of flowers from the same cultivar before extraction were on average 134 g/kg of total CBD and 420 mg/kg of total THC in air DM. The hemp plants were grown to the flowering stage in a greenhouse with supplemental lighting and irrigated with a nutrient solution. After harvest, the dried and ground hemp flowers had been subjected to a laboratory-scale aqueous ethanol extraction for cannabinoid removal, following a standard solid-liquid extraction approach commonly used for analytical cannabinoid assessment (e.g., Citti et al., 2016). After extraction under agitation the solvent was removed, and the resulting residue was air-dried and ground to pass a 0.5 mm sieve. Authorization to feed this hemp residue to hens was obtained from the responsible Swiss authority (Agroscope, Posieux, Switzerland) under the condition that the foods produced were excluded from the human food chain. The complete experimental diets were obtained by mixing the ground materials in a 100 kg shaft feed mixer (Gericke, Zurich, Switzerland) for 15 min in the proportions intended (Table 1) and served as mash. Sieve analysis confirmed a similar particle size of all three feeds, thus minimizing the hens' component selection by particle size.

2.3. Experimental schedule

An a priori power analysis was performed using the pwr4exp package in R (version 1.0.1, R Core Team) following Wang et al. (2025), with energy metabolizability (scale: 0 to 1) as the primary response variable to describe feeding value. The alfalfa diet, analyzed in advance to contain 170 g/kg more aNDFom than the control diet, was expected to reduce energy metabolizability by at least by 0.1 of total. The mean response for the hemp residue diet was assumed to be either similar to the alfalfa diet or intermediate between the control and alfalfa diets. Variance was set at 5×10^{-4}, corresponding to the highest value reported in previous studies carried out with the same equipment and measurement technique (Gangnat et al., 2020; Heuel et al., 2021, 2022). With 15 hens per group, the design provided an estimated power of ≥ 0.9 to detect differences between treatments and control at $\alpha = 0.05$.

Accordingly, three groups of 15 hens each, Control, Alfalfa and Hemp, were formed following a randomized block design balancing groups for average BW of the hens. The experiment lasted for 37 days (Fig. 1). To familiarize the hens with green feed color, week 1 served as an adaptation period during which all hens received, per kg of diet, a mixture of 900 g of basal feed and 100 g of alfalfa. Afterwards, the Control and Alfalfa groups stayed on this diet until the start of the data and sample collection period on the last 12 days of the experiment (days 26 to 37). In that period the Control group received exclusively the basal feed, and in the Alfalfa group the dietary alfalfa proportion was raised to 150 g/kg, an inclusion level similar to other recent studies (Irawan et al., 2024, 2026) and known to enhance yolk coloration without impairing performance (Laudadio et al., 2014). In the Hemp group, alfalfa was gradually replaced by hemp flower residue until reaching 150 g/kg of the total diet in the main data and sample collection period. The approach of adding alfalfa instead of including it in the complete feed followed common practice on various farms. Accordingly, all macro- and micro-nutrients of the basal diet were diluted by adding alfalfa and hemp flower residue, but this to the same degree thus facilitating the comparison of the two supplemented feeds. Table 1 gives the ingredient composition of the experimental diets fed in the main data and sample collection

period. On the last 9 days of the experiment (days 29 to 37), five randomly chosen hens fed until day 28 like the Hemp group of 15 hens were subjected to a washout period by providing them with a diet where hemp flower residue was completely replaced by alfalfa (Washout group). This washout schedule allowed assessment of whether, and to which extent, the effects of hemp feeding were reversible within this period. This period of 9 days was arbitrarily chosen due to the lack of references on poultry, previous studies focusing on mammals. A 9-day period corresponds to twice the 4 to 5-day period reported in mammals for substantial reduction (half-life or even lesser concentration) of CBD and THC (Consroe et al. 1991; Fruge et al., 2025). This expected reduction in CBD and THC concentration also justifies only 5 hens were estimated necessary for the Washout group. Further, a 9-day period of washout would be applicable on farm (rearing hens, broilers and, with restrictions, layers). All hens were slaughtered on day 37.

2.4. Data collection and laboratory analyses

2.4.1. Data and sample collection

Group allocation of the hens was color-coded, so that the animal caretakers worked in a blinded manner. Yet the typical smell of the hemp was obvious in the hemp-containing feed. At least twice daily, health status (feed intake, feather condition, activity, form of the droppings) of the hens was visually monitored. Individual feed consumption was determined twice weekly (Monday and Friday). Body weight was measured once a week. Diet samples (control, 150 g/kg alfalfa and 150 g/kg hemp) were taken during the last experimental week. Eggs were collected and weighed daily. Excreta were collected from day 30 through day 32 (except in the Washout group), immediately frozen at –20°C, then lyophilized and ground with a centrifugal mill to pass a 0.5 mm screen. At slaughter (day 37), blood was sampled in Na-heparin tubes and put on ice until centrifugation at 2,300 *g* at 4°C for 10 min. The plasma was then stored at –20°C. Both breast muscles were removed, homogenized by Moulinette grinder and frozen at –20°C in vacuumed plastic bags in portions of about 10 g/bag.

2.4.2. Proximate analysis of feed and excreta

Test feeds and complete diets were analyzed for proximate contents (excreta only DM, nitrogen and gross energy; this to be able to calculate metabolizability indices) following standard procedures (AOAC, 1997). Contents of DM and total ash were analyzed by an automatic thermo-gravimetric device (model TGA-701, Leco, St. Joseph, MI). Organic matter was calculated as the difference between the two variables. Crude protein was determined as 6.25 × nitrogen, where nitrogen had been assessed by a C/N analyzer (model TruMac® CN, Leco, St. Joseph, MI; AOAC index #968.06). Ether extract was determined with a Soxhlet extraction system (B-811, Büchi, Flawil, Switzerland; AOAC index #963.15). Neutral detergent fiber (aNDFom), corrected for ash content, was analyzed on a Fibertec System M (Tecator, 1020 Hot Extraction, Foss, Hillerød, Denmark) according to Mertens (2002) using 100 µL of α-amylase (Sigma-Aldrich, St. Louis, USA). A bomb calorimeter was used to determine gross energy (Calorimeter System C700 with Cooler C7002, IKA, Staufen, Germany). The 4 M-HCl insoluble ash content was analyzed in diets and excreta according to Vogtmann et al. (1975).

2.4.3. Egg quality analysis

The eggs from day 34 (day 35 for two hens that did not lay on day 34) were collected to assess different egg properties. Egg weight and shell strength were measured with an electronic hardness tester (type PTB 301, Pharma Test, Hainburg, Germany). Yolk and shell were weighed, the latter after cleaning and drying for 24 h at 50°C. Albumen weight was calculated as the difference between total egg weight and the sum of yolk and shell weights. Yolk color was determined in the L*a*b* color space using a chromameter (model CR-300, Minolta, Ramsey, NJ, USA). Afterwards, albumen height was measured at 1 cm from the yolk. Haugh units were then calculated as $100 \times \log [\text{albumen height (mm)} - 1.7 \times \text{egg weight (g)}^{0.37} + 7.6]$ (Haugh, 1937).

2.4.4. Cannabinoid analysis

Analysis of CBD, CBDA, THC and THCA in the hemp flower residue was performed by Puregene AG using a method adapted from Storm et al. (2020). In detail, cannabinoid extraction from hemp flower residue was performed in triplicate first applying mechanical homogenization in a VWR Starbeater mill. Approximately 500 mg of material (weight noted) and 15 ml ethanol (0.996 of total, Ph.Eur. grade) were put in a disposable 50 ml test tube with zirconia beads (~2 mm diameter). Cannabinoids were extracted by shaking for 5 min at 25 Hz. An aliquot of the crude extract was directly filtered through a 0.2 µm PTFE syringe filter and diluted as needed with ethanol. The cannabinoid assay was run on a 1290 Infinity II HPLC system (Agilent Technologies, Santa Clara, CA) equipped with Diode Array Detector (DAD), temperature-controlled column compartment, multisampler, and quaternary pump. The separation of the analytes was achieved on a Kinetex 1.7 µm EVO C18 100A 100 × 1.2 mm column (Phenomenex, Torrance, CA). Full spectra were recorded from 200 to 400 nm, and absorbance at 230 nm was used to quantify cannabinoids. Instrument control, data acquisition, and integration were achieved with OpenLAB CDS (Agilent Technologies) software, applying an identification and quantification method based on a 5-level external standards calibration curve. To confirm the analyte identity in plant material, retention time and peak purity were compared with the signal acquired on certified reference materials. The calibration curve used for quantification of the most common cannabinoids was obtained by analyzing serial dilutions of cannabinoid mixtures produced in house from commercially available cannabinoids (Supelco, Merck&Cie, Buchs, Switzerland), namely, CBD, acidic CBD (CBDA), Δ9-THC, and acidic Δ9-THC (THCA). The concentration of cannabinoids was calculated in relation to the air DM of hemp flower residue (w/w).

Concentrations of CBD, CBDA, THC and THCA were analyzed in blood plasma and breast muscle using a validated liquid chromatography tandem-mass spectrometry (LC-MS/MS)

method developed for whole blood (Haas et al., 2023), expanded and adjusted for CBDA and THCA. Validation included THC, CBD, HO-THC, and THC-COOH in whole blood according to the guidelines of the Society of Toxicological and Forensic Chemistry (GTFCh), including calibration model and limits of quantification, accuracy, intra- and inter-day imprecision, recovery, matrix effects, and stability. All parameters met the acceptance criteria (proportion of total): calibrator accuracy <0.3; bias and imprecision <0.15 (<0.2 at the lowest concentration); recovery >0.5; matrix effect <0.3. The validated method was applied unchanged to muscle tissue samples; no additional matrix-specific recovery experiments were conducted because conventional spiking studies do not adequately assess analyte extraction from intact tissue, and the extraction procedure has previously been extensively evaluated in postmortem tissue analysis (Staehli et al., 2015). Cannabinoid extraction from blood plasma samples was accomplished by simple protein precipitation (PP). Briefly, 200 µL plasma was mixed with 50 µL of a heavy-labelled internal standard mixture (IS, THC-d3, CBD-d3), and PP was performed by slowly adding ice-cold acetonitrile (500 µL) during vortexing. The mixture was vortexed, shaken for 10 min (1400 rpm), and centrifuged (5 min 10620 × *g*). The supernatant was transferred into an autosampler vial, evaporated to dryness under a gentle stream of nitrogen at ambient temperature, and reconstituted in 60 µL of a mixture of mobile phases A and B (1:1, v/v). From 100 mg of breast meat tissue cannabinoids were extracted by first spiking it with 50 µL IS and mixed with 500 µL phosphate buffer (0.2 M, pH 7.4), followed by 1 mL of a butyl acetate/ethyl acetate mixture (1:1, v/v) as extraction solvent. Homogenization and liquid-liquid extraction were performed simultaneously using a Fast Prep ® 24 instrument (MP Biomedicals, https://www.mpbio.com/sg), followed by shaking (10 min, 1,400 rpm) and centrifugation (10 min, 10,620 × *g*). The supernatant was transferred into an autosampler vial, while the remaining sample was again liquid-liquid-extracted (1 mL extraction solvent) after the addition of 200 µL 1 M NaOH. The combined supernatants were evaporated to dryness under a gentle stream of nitrogen at ambient temperature and were reconstituted in 60 µL of a mixture of mobile phases

A and B (1:1, v/v). No hydrolysis had been made prior to sample preparation. Extracts were then analyzed for cannabinoids by liquid chromatography tandem-mass spectrometry (LC-MS/MS) on a Shimadzu Nexera HPLC/UHPLC LC 40D XR (Shimadzu, Kyoto, Japan) coupled to a Sciex TripleQuad 7500 equipped with an OptiFlow Pro Ion Source (Sciex, Framingham, USA). The liquid chromatography settings were as follows: Phenomenex (Aschaffenburg, Germany) Kinetex C18 (1.7 µm, 100A, 100 mm × 2.1mm), gradient elution with 10 mM ammonium formate buffer in water containing 10 mL acetonitrile and 1 mL/L (v/v) formic acid (eluent A) and acetonitrile containing 10 mL H_2O and 1 mL/L (v/v) formic acid (eluent B); injection volume 10 µL. The gradient (total run time 15 min, flow rate 0.5 mL/min) was 0-1.5 min(A:B, 0.6:0.4), 1.5-1.6 min to (A:B, 0.4:0.6), 1.6-10 min to (A:B, 0.35:0.65), 10-11 min to (A:B, 0.05:0.95), 11-12 min hold, 12.1 min return to start and re-equilibrate for 3 min. The OptiFlow Pro Ion Source was operated in positive electrospray ionization (ESI) mode with the following mass spectrometry conditions: gas 1, nitrogen (45 psi); gas 2, nitrogen (60 psi); ion spray voltage, 1750 V; ion-source temperature, 250 °C; curtain gas, nitrogen (32 psi), collision gas 9. The MS was operated in the scheduled multiple reaction monitoring mode using two transitions per analyte and one transition per IS, respectively, and controlled by Sciex OS®. Quantification was performed in SciexOS® against a seven-point calibration curve (1-100 µg/L or µg/kg). Accordingly, the limits of quantification were 1 µg/L blood plasma and 1 µg/kg breast muscle. The limit of detection was not systematically evaluated. Each analytical batch was controlled by analyzing three independently prepared quality control samples covering the entire calibration range.

2.5. Calculations and statistical analysis

One hen each was missing in Control and Alfalfa groups because both animals started molting early in the experiment. The feed efficiency was calculated as a ratio of feed consumption to egg mass produced. The diets had been supplemented with 1.6 g celite/kg, an

indigestible and acid insoluble indicator, to increase the accuracy of the calculation of the apparent energy and nitrogen metabolizability. This calculation was accomplished as outlined by Vukić Vranješ et al. (1994) with the help of the known intake of acid-insoluble ash. Total CBD was calculated according to the following formula: CBD (mg/kg) + CBDA × 0.877 (mg/kg), with the factor of 0.877 accounting for decarboxylation of the CBDA molecule (Stevens et al., 2022). Similarly, total THC was calculated as Δ9-THC (mg/kg) + Δ9-THCA × 0.877 (mg/kg).

Statistical evaluation was performed with the GLM procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Dietary treatment effects were determined by ANOVA. The individual hens were the experimental units (n=14, 14 and 15 for Control, Alfalfa and Hemp groups, respectively). Normal distribution of the residuals was evaluated with the Univariate procedure and Q-Q plot of the residuals. Results are given as least square means ± SEM. Multiple comparisons among means were performed using the Tukey-Kramer adjustment method and $P < 0.05$ was considered statistically significant. Significance of the differences between the Hemp group (n=15) and the Washout group (n=5) regarding cannabinoid concentrations in plasma and muscle were determined identically, except that the data were log transformed before analysis. Values below detection level were set to zero and 1 was added to each value before log transformation. The results of these comparisons are given as arithmetic means ± standard deviation.

3. Results

The dietary inclusion of either 150 g/kg of alfalfa or hemp had no effect ($P > 0.10$) on feed intake, final BW, laying performance (eggs/day, egg weight and egg mass yield), and feed efficiency (Table 2). The overall laying performance was maintained at around 0.95 eggs/day in all groups. This, despite the lesser ($P < 0.001$) intake of metabolizable energy in the Hemp

group compared to the Control group. The difference in intake of metabolizable energy between the Alfalfa group and the Control group almost reached significance ($P = 0.0502$).

The proportions of yolk, albumen and shell were not affected ($P > 0.10$) by the inclusion of alfalfa or hemp (Table 2). Lightness (L*) and redness (a*) of the yolk differed among groups ($P < 0.001$). Egg yolks from the Hemp group were darker (lower L*) and redder (higher a*) than eggs from the Control group ($P < 0.05$); eggs from the Alfalfa group had intermediate values. The increase in yolk redness in the Hemp group was substantial, with values being 2.7-fold higher than those of the Control group. Yellowness (b*) remained unaffected by diet. Haugh units, an indicator of the processing quality of the albumen, showed a weak tendency ($P < 0.10$) to be lower by 3 to 4 Haugh units in the Hemp group compared to the Control group. Still all values remained within the range acceptable for table eggs.

In the hemp flower residue, and consequently in the diet containing 150 g hemp/kg, the concentrations of total CBD (122 mg/kg air-DM; including its acidic form, CBDA) were much higher than those of total THC (6 mg/kg air-DM; including its acidic form, THCA) (Table 3). In both cannabinoids, the acidic forms made up a higher proportion than the respective decarboxylated cannabinoids. The CBD:THC ratio was 20:1. All four cannabinoids analyzed were detected in plasma and muscle of hens in the Hemp group, reflecting the basic differences in concentrations of individual cannabinoids in the hemp diet. The average plasma concentrations of total CBD and total THC were about 3,400 and 400 μg/L, respectively (CBD:THC ratio of 8:1), and they were 139 and 47 μg/kg in the muscle, respectively (CBD:THC ratio of 3:1). After 9 day of washout, all forms of cannabinoids were still detected in plasma of all five hens with average concentrations of total CBD and total THC being 135 and 7 μg/L (CBD:THC ratio of 19:1), respectively. In the muscle of the Washout group, CBDA and THC were above detection limit in four and two of five hens, respectively, and CBD and THCA were not detected. Average total CBD and total THC levels in muscle across all five

hens were about 8.2 and 0.2 μg/kg. All cannabinoid values measured in the hens of the Washout group clearly differed ($P < 0.001$) from that of the hens fed the hemp diet continuously.

DISCUSSION

4. Discussion

4.1. Feeding value of hemp flower residue

Diet formulation had been made to facilitate especially the comparison of hemp flower residue with alfalfa. The comparison of the feeding value of these two supplemented feeds with that of the basal diet is limited by the dilution of the macro- and micronutrients. The results of the present study point towards a similar or even slightly superior feeding value of hemp flower residue compared with that of alfalfa, at least in the condition of the present study using few laying hens per group, at the end of lay, under optimal experimental conditions and over a short period. When included at 150 g/kg of the diet, the hemp flower residue did not impair palatability, as the intake of the hemp diet did not differ from that of the other diets. Moreover, the hens obviously readily adapted to the green color of the feed at the beginning of the experiment, suggesting that color had little or no impact on feed acceptance. There was no significant difference between the hemp diet and the alfalfa diet in intake, BW, performance, feed efficiency, metabolizability, and most variables of egg quality. Compared to alfalfa, the hemp flower residue appeared to promote yolk color intensity. Although the differences in yolk darkness and redness did not reach statistical significance, the direction of change aligns with the higher pigmentation potential of hemp material. Furthermore, the hemp flower residue was clearly richer in CP (1.7-fold content of that of alfalfa), a level being almost as high (236 g/kg DM) as the 250 g CP/kg DM reported by Kleinhenz et al. (2020a) for extracted hemp flower. The hemp flower residue also contained less fiber (NDF) than alfalfa (263 vs. 473 g/kg DM). Kleinhenz et al. (2020a) noted 310 g NDF/kg in DM for the extracted flower which is slightly higher. Considering the large differences in CP and NDF content, it remains puzzling why the

use of the hemp diet did not result in a better metabolizability (i.e., improved metabolic protein and energy supply) than the alfalfa diet. This suggests that other characteristics of the residue – such as fiber composition, particle structure, or residual bioactive compounds – may have limited the apparent utilization of nutrients despite the favorable proximate profile.

As expected, diets supplemented with alfalfa and hemp flower residue, both diluting macro- and micronutrients of the basal compound feed, resulted in a reduced metabolizability of dietary energy and nitrogen and intake of metabolizable energy. Performance was no reliable indicator of the comparative feeding value here as the hens were in late stage of lay (though with astonishingly high laying performance) and the time period of the different diets could have been too short to promote performance differences. The results of the present study suggest that hemp flower residue, like alfalfa, functions more as a roughage-type feed than a concentrate-type feed. This distinguishes it from hempseed-based feeds (full-fat, meal, oil), which have energy or protein contents equivalent to or higher than those of a complete compound feed for laying hens (e.g., Shariatmadari, 2023; Ötztürk et al., 2024). Still, strategically used, the hemp flower residue might be useful to complement low-CP diets, although confirmation of this would require further evaluation of the protein quality (amino acid composition). Additionally, the enhanced yolk redness observed in the Hemp group suggests that hemp flower residue may serve as a natural yolk colorant, which could hold practical value in production systems and markets where deeper yolk pigmentation from natural sources is preferred.

Published research on the use of hemp plants or plant parts in poultry nutrition has so far focused almost exclusively on materials derived from hempseed – a co-product primarily of hemp fiber production – because in many countries these are the only hemp-based materials currently permitted for use as animal feed. The studies with laying hens include hempseed meal/cake (oil widely removed by extraction/pressure), pure hempseed oil, and full-fat hempseed (recently reviewed by Fallahi et al., 2022; Shariatmadari, 2023, Altmann et al., 2024, Lanzoni et al., 2024, as well as Neijat and House, 2024). Hempseed meal and cake are valuable

protein sources, exceeding 300 g CP/kg (Shariatmadari, 2023; He et al., 2025) and are as such suitable to replace soybean meal in layer diets (e.g., Öztürk et al., 2024). In contrast, hempseed oil has a favorably high proportion of n-3 polyunsaturated fatty acids and a low n-6/n-3 fatty acid ratio, thus being superior to lipids from various other oil seeds (Wang et al., 2017). Hempseed oil and full-fat hempseed may therefore improve the human health value of the egg lipids, and they are valuable energy sources in addition (e.g. Gahkar et al. 2012; Jing et al., 2017). Going beyond pure hempseed-based products, Lanzoni et al. (2025) tested in layers a hemp co-product obtained from the cleaning of entire hempseed, which included leaves, non-standard hempseed, hempseed hulls and stems. Balenović et al. (2024) tested the effects of hemp leaves on health-related traits in broilers. The only poultry study performed with hemp flower residue (here: spent hemp biomass, also a residue of cannabinoid extraction) was that of Parker et al. (2026) in fast growing broilers. The authors reported unchanged feed intake but reduced growth performance from 100 g hemp material/kg diet although being isocaloric and isonitrogenous to the control diet. The present results in laying hens at the end of lay contrast with the results of Parker et al. (2026). This highlights the need for further research in younger hens, in hens facing environmental challenges or on a longer feeding period than in the present study.

4.2. Concentrations of cannabidiol and tetrahydrocannabinol in hemp flower residue, plasma and muscle

4.2.1. Cannabinoids in the extracted hemp flowers

Unextracted industrial hemp flower of conventional hemp cultivars was described to contain about 30 g total CBD and 4 g total THC/kg (Kleinhenz et al., 2020a). The unextracted flower of high-CBD-low-THC hemp cultivars can even contain up to 200 g/kg total CBD and up to 10 g/kg THC (Puregene AG – Swiss retail hemp flower study, unpublished). The unextracted material of the cultivar used in the present study was found to have a total CBD

concentration in that range (134 g/kg), whereas total THC was clearly lower (0.4 g/kg). For extracted flower, Kleinhenz et al. (2020a) listed levels of 10 g total CBD and 0.3g total THC/kg material. In the present study the ethanol extraction of cannabinoids from CBD-hemp flower material lowered total CBD and total THC concentrations to much lower levels of 0.1 and 0.006 g/kg, respectively. Considering the known large differences in cannabinoid concentrations in spent hemp biomass obtained from different processors (Irawan et al, 2025a), the extraction procedure applied to the present material turned out to be very efficient.

Currently, the European Commission (2025) is discussing upper limits for THC in feed material from hemp. Thresholds suggested are < 3 mg THC/kg air DM for hempseed and expeller and < 7.5 mg THC/kg air DM for hemp oil, flour and fiber. Under these conditions, hemp-based feeds would be allowed to be included in layer diets at levels that do not exceed 0.5 mg/kg of total THC. The THC concentration of the present hemp flower residue (6.2 mg/kg air-DM) falls below the threshold of 7.5 mg/kg air DM, indicating that the material could possibly meet the criteria as a feed ingredient. However, to obey the European Commission (2025) limit for the entire diet, the dietary inclusion rate would need to be capped at 80 g rather than the 150 g/kg of hemp flower residue used in the experimental diet.

According to Russo and Marcu (2017), CBD and THC are present in hemp almost exclusively in their acidic forms (CBDA and THCA), but Kleinhenz et al. (2020a) found the decarboxylated forms in hemp flower as well, although at much lower proportions than those of the acidic forms. Consistent with this, in the present hemp flower residue the acidic forms were also much higher in concentration than the decarboxylated forms (CBDA:CBD, 21:1; THCA:THC, 3:1). The acidic forms (CBDA and THCA) are pharmacologically not the final active forms but are readily absorbed after oral intake (Wakshlag et al., 2020). The decarboxylated (neutral) forms (CBD and THC) instead are pharmacologically directly active, but their intestinal absorption is more limited (Wakshlag et al., 2020). The transformation of the acidic to the neutral forms is accomplished by decarboxylation. This transformation may

occur naturally as the plant ages or when the material is held in storage Goffman et al. (2025) and is exposed to light or heat (e.g., baking, smoking, cooking; Russo and Marcu, 2017).

4.2.2. Accumulation in plasma and muscle after 30 days of hemp feeding

The Hemp group received the hemp flower residue during 30 days (dietary proportions g/kg: 10 for 4 days, 50 for 7 days, 100 for 7 days, and 150 for 12 days; equaling to about 100 g/kg on average across the 30 days). This schedule resulted in proportionate concentrations of total CBD equivalent to 0.2 (plasma) and 0.007 (muscle) of those present in the complete diet; the corresponding proportions for total THC were 0.4 and 0.04, respectively. Results on cannabinoid concentration in plasma and meat when feeding hemp-based material are only available from ruminants. Smith et al. (2023) fed crossbred heifers a diet with 200 g hempseed cake/kg (0.26 mg of (assumed) THC/kg) for almost 4 months and detected none of these cannabinoids in plasma and muscle. Spent hemp biomass, also a residue from CBD extraction, fed for 8 weeks either at 200 g/kg diet to lambs (Irawan et al. 2024) or at 150 g/kg diet to beef steers (Irawan et al., 2026) resulted in either 0.63 μg/kg of THC or the lack of THC in the muscle, respectively. This meant far lower concentrations than the level of decarboxylated THC found in the hens of the present experiment. Feeding hemp leaves to beef steers at a rate corresponding to an intake of 3.9 mg CBDA/kg BW resulted in peaks in plasma of about 250 μg of CBDA and 20 μg THCA/L 12 h after the start of sampling, with negligible concentrations of CBD and THC (Fruge et al., 2025). This is higher than the 139 μg/kg total CBD but lower than the 47 μg/kg total THC found for both cannabinoids in the hens' muscle tissue. Comparisons with earlier studies on the transfer of cannabinoids from feed to ruminant tissues (e.g. Krebs et al., 2021; Stevens et al., 2022) should be made with caution due to differences in plant material (hemp stubble, hemp hay), cannabinoid concentrations, and analytical methodologies. Smith et al. (2023) estimated that the consumption of beef adipose tissue containing 10 μg/kg THC was unlikely to exceed the acute reference dose recommendations

given by various organizations, among them EFSA (2015). Accordingly, eating breast muscle from the hemp-fed hens would likely also fall below thresholds of toxicological concern.

Overall, it is striking that – compared with the hens in the present study – ruminants appear to exhibit lower plasma concentrations and low to undetectable muscle concentrations at the same or even higher dietary cannabinoid concentrations. This suggests that in ruminants there is a lower systemic availability of cannabinoids than in poultry, either due to degradation in the rumen or reduced intestinal absorption. Concerning adipose tissue, the results found in ruminants (e.g., Stevens et al., 2022, Smith et al., 2023; Fruge et al., 2025) indicate that cannabinoid accumulation is more pronounced than in lean as cannabinoids are fat soluble. This suggests that carcass fat and eggs of the hens of the present study also may have contained more cannabinoids than the lean breast muscle and maybe even more than the plasma, and the levels might exceed the acute reference dose recommendations mentioned above. Further studies have to determine the extent of permeability of the barrier from blood to uterus to egg for cannabinoids. No other data on cannabinoids in eggs appear to be available – even since EFSA stated in 2011 that this would be the case. Promising in this context is the absence of THC and CBD in the milk of spent-hemp biomass fed cows where obviously the blood-mammary gland barrier effectively blocked any transfer (Irawan et al., 2025b).

Another notable finding of the present study was that the relative CBD:THC ratio narrowed progressively from feed (20:1) to plasma (8:1) to muscle (3:1), indicating proportionally greater accumulation of THC than CBD at the level of digestion and again when being deposited in tissues. The higher abundance of the acidic compared to the decarboxylated forms of CBD and THC in the muscle observed likewise is probably of limited relevance for toxicological assessment of potential adverse effects from consuming such feeds, because cooking would decarboxylate these compounds and thus contribute to overall cannabinoid exposure.

4.2.3. Efficiency of a 9 day washout period

The rather short time of washout used in the present study was owed to the practicability of such a measure on farm. The Washout group had received the hemp flower residue for 21 days (dietary proportions g/kg: 10 for 4 days, 50 for 7 days, 100 for 7 days, and 150 for 3 days). For both CBD and THC, the values found in plasma and muscle were clearly lower than those in the Hemp group. In detail, the plasma levels measured in the Washout group suggest a depletion to proportionately approximately 0.04 (CBD) and 0.02 (THC) of the concentrations measured in the continuously hemp-fed hens. In the breast muscle, the values found in the Washout group corresponded to reductions to proportionately 0.06 and <0.01 for CBD and THC, respectively. This shows that even a short dietary withdrawal markedly depletes cannabinoid muscle stores, but clearance was still incomplete in some individuals. The only other study in non-ruminant farm animals, in which pigs were used as a model to investigate THC kinetics (Brunet et al., 2006) showed that the short-term depletion of THC was fastest in the liver, intermediate in the muscle, and slowest in the adipose tissue. Recent studies in ruminants using hemp-derived residues and comparable matrices provide further context for cannabinoid accumulation and persistence. In particular, Irawan et al. (2026) demonstrated that CBD and Δ^9-THC were low but remained detectable in muscle tissue even after 2 weeks of washout, highlighting the persistence of cannabinoids when hemp residues are used as feed. Similarly, recent analytical and exposure-focused studies have reported prolonged retention of cannabinoids in animal tissues and emphasized the role of lipid-rich compartments as reservoirs for these compounds (Irawan et al., 2024, 2025b, 2026). These findings support the interpretation that cannabinoid accumulation and depletion kinetics depend strongly on the type of hemp material used, its cannabinoid profile, and the physiological characteristics of the animal. In this context, comparisons with ruminants and with earlier studies using different hemp material should be made with caution due to differences in animal physiology, plant material, cannabinoid concentrations, and analytical methodologies.

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4.2.4. Limitations and aspects of future studies

Lacking reference studies on birds, the washout period of 9 days was chosen arbitrarily based on previous studies in mammals reporting 4 to 5 days to substantially reduce blood levels of CBD and THC but several weeks for adipose tissue (Consroe et al. 1991; Fruge et al., 2025; Irawan et al., 2025b, 2026). The present study successfully reports lesser levels in plasma and muscle concentration of CBD and THC after a 9-day washout period. Future studies will have to investigate the clearance kinetics in detail in poultry, including adipose tissue and egg. It remains open whether a 5-day washout period would have been sufficient to obtain this result, whether a longer feeding period and/or greater inclusion level also imply a longer withdrawal period, and how far adipose tissue mobilisation influences blood, muscle and egg concentration in CBD and THC. Adipose tissue mobilisation was indeed suggested as the reason for the rise in CBD in milk at the beginning of lactation in the study of Irawan et al. (2025b).

5. Conclusions

Hypothesis a) stating that the feeding value of the hemp residue is at least as high as alfalfa meal was confirmed. The residue was even slightly superior in its egg yolk coloring property. Hypothesis b) claiming that CBD and THC can be detected in blood after absorption from the gut, but appear only in low or undetectable concentrations in edible tissues, was only partially confirmed in the present study. Levels in blood were indeed clearly higher than those in muscle tissue, but both cannabinoids were still detected in muscle. Based on its cannabinoid profile, hemp flower residue therefore may represent a potential feed material; however, conclusions regarding safety for consumers cannot be drawn from the present study, as a formal risk assessment was beyond its scope. In particular, the persistence of cannabinoids in tissues and the potential for accumulation in lipid-rich matrices such as egg yolk and adipose tissue warrant further investigation. Future studies should integrate exposure-based risk assessment approaches, such as those proposed in recent literature and by EFSA, to better evaluate

consumer safety. Hypothesis c) stating that already a 9-day washout period is sufficient to deplete THC and CBD in muscle to levels below the detection limit was also only partially confirmed as cannabinoids were still detected in plasma of all hens and in muscle of some individuals. If hemp flower residue were to be allowed as feedstuff only under the requirement that food products should be free of cannabinoids, its use in the poultry sector would be restricted to the starting phase in broilers or the rearing phase in layers as a washout period is less applicable during egg production.

Ethics declaration

Animal subject

This study was conducted in accordance with the following guidelines for animal welfare and/or reporting: Authorization from the cantonal veterinary office of Zurich, Switzerland (license no. 221/17).
This study was approved by the Cantonal veterinary office of Zurich, Switzerland.
(Approval No. 221/17)

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CRediT author contribution statement

Isabelle Gangnat: Conceptualization, Methodology, Data analysis, Investigation, Data curation, Writing – review and editing, Project management. **Andreia Arbenz:** Methodology, Data analysis, Data curation, Writing – review and editing. **Andrea E. Steuer:** Methodology, Data curation, Writing – review and editing. **Leron Katsir:** Methodology, Data curation, Writing – review and editing. **Michael Kreuzer:** Writing – original draft preparation, Supervision.

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Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

AAFCO (Association of American Feed Control Officials). 2024. Hemp as a Feed Ingredient; a National Discussion. https://www.aafco.org/news/hemp-as-a-feed-ingredient-a-national-discussion/. Accessed on 15 April 2026.

Altman, A.W., Kent-Dennis, C., Klotz, J.L., McLeod, K.R., Vanzant, E.S., Harmon, D.L., 2024. Review: Utilizing industrial hemp (Cannabis sativa L.) by-products in livestock ration. Anim. Feed Sci. Technol. 307, 115850. https://doi.org/10.1016/j.anifeedsci.2023.115850.

AOAC (Association of Official Analytical Chemists), 1997. Official Methods of Analysis. 16th ed., Washington, DC.

Balenović, M., Janječić, Z., Savić, V., Kasap, A., Popović, M., Šimpraga, B., Sokolović, M., Bedeković, D., Kiš, G., Zglavnik, T., Špoljarić, D., Krstulović, F., Listeš, I., Zelenika, T.A., 2024. Immunostimulatory and antibacterial effects of *Cannabis sativa* L. leaves on broilers. Animals 14, 1159. https://doi.org/10.3390/ani14081159.

Brunet, B., Doucet, C., Venisse, N., Hauet, T., Hébrard, W., Papet, Y., Mauco, G., Mura, P., 2006. Validation of large white pig as an animal model for the study of cannabinoids metabolism: application to the study of THC distribution in tissues. Forensic Sci. Int. 161, 169–174. https://doi.org/10.1016/j.forsciint.2006.04.018.

Citti, C., Ciccarella, G., Braghiroli, D., Parenti, C., Vandelli, M.A., Cannazza, G., 2016. Medicinal cannabis: Principal cannabinoids concentration and their stability evaluated by a high performance liquid chromatography coupled to diode array and quadrupole time of flight mass spectrometry method. J. Pharmaceut. Biomed. Anal. 128, 201–209. https:// doi.org/10.1016/j.jpba.2016.05.033.

Consroe, P., Kennedy, K., Schram, K., 1991. Assay of plasma cannabidiol by capillary gas chromatography/ion trap mass spectroscopy following high-dose repeated daily oral administration in humans. Pharma. Biochem. Behav. 40, 517–522. https://doi.org/10.1016/0091-3057(91)90357-8.

EAER (Swiss Federal Department of Economic Affairs, Education and Research), 2023. Ordinance of the WBF (Federal Office for Agriculture) on the Production and Marketing of Feed, Feed Additives and Dietetic Feed. Appendix 4.1, p. 417 (in German). https://www.lexfind.ch/tolv/254172/de.

EFSA (European Food Safety Authority; Panel on Additives and Products or Substances used in Animal Feed). 2011. Scientific Opinion on the Safety of Hemp (*Cannabis* genus) for Use as Animal Feed. EFSA J. 9, 41 pp. https://doi.org/10.2903/j.efsa.2011.2011.

Journal Pre-proof

EFSA (European Food Safety Authority; Panel on Contaminants in the Food Chain). 2015. Scientific Opinion on the Risks for Human Health Related to the Presence of Tetrahydrocannabinol (THC) in Milk and Other Food of Animal Origin. EFSA J. 13, 4141. https://doi.org/10.2903/j.efsa.2015.4141.

European Commission, 2025. Initiative on 'Animal Feed – Setting and Reviewing Maximum Levels of Certain Undesirable Substances in it. https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/14098-Setting-and-reviewing-maximum-levels-for-certain-undesirable-substances-in-feed_en. Accessed on 15 April 2026

European Union, 2013. Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of Feed Materials. Official J. Europ. Union L 29, 1–64. https://eur-lex.europa.eu/eli/reg/2013/68/oj/eng.

European Union, 2021. Regulation (EU) 2021/2115 of the European Parliament and of the council of 2 December 2021. https://eur-lex.europa.eu/eli/reg/2021/2115/oj/eng.

Fallahi, S., Bobak, Ł., Opaliński, S., 2022. Hemp in animal diets—cannabidiol. Animals 12, 2541. https://doi.org/10.3390/ani12192541.

Fruge, G., Magnin, G., Jessup, R., Bruner, B., Kleinhenz, M., Drewery, M.L., 2025. Cannabinoid transference and retention in tissues of beef steers fed hemp leaves. Food Additives Contamin. A, 42, 703–715, https://doi.org/10.1080/19440049.2025.2506106

Gakhar, N., Goldberg, E., Jing, M., Gibson, R., House, J.D., 2012. Effect of feeding hemp seed and hemp seed oil on laying hen performance and egg yolk fatty acid content: Evidence of their safety and efficacy for laying hen diets. Poult. Sci. 91, 701–711. https://doi.org/10.3382/ps.2011-01825.

Goffman, F.D, Carrera, D.A., Latino, D.A.R.S., Cronje, C., Katsir, L., 2025. Impact of electron beam treatment and storage duration on microbial stability and phytochemical integrity in hemp flowers. Molecules 30, 3601. https://doi.org/10.3390/molecules30173601.

Haas, C., Salzmann, A.P., Binz, T.M., Staubli, G., Seiler, M., Steuer, A.E., 2023. Analytical description of adolescent binge drinking patients. BMC Pediatr. 23, 512. https://doi.org/10.1186/s12887-023-04325-2.

Haugh, R.R., 1937. The Haugh unit for measuring egg quality. US Egg Poult. Magazine 43, 552–555.

He, Q., Zhang, T., Tian, G., Wang, H., Lu, X., Deng, H., Yang, F., Tang, X., Wang, J., Li, Z., Li, H., Shen, S., Lu, Y., Huang, J., 2025. Effects of partial replacement of soybean meal with hemp seed (*Cannabis sativa* L.) cake on the growth and meat quality in female three-yellow chickens. Poult. Sci. 104, 104466. https://doi.org/10.1016/j.psj.2024.104466.

Gangnat, I.D.M., Mueller, S., Messikommer, R.E., Kreuzer, M., 2020. Performance, egg quality and resilience to nutritional challenges of Lohmann Dual hens as opposed to layer and traditional dual-purpose types: a preliminary study. Europ. Poult. Sci. 84, 16 pp. https://doi.org/10.1399/eps.2020.301.

Heuel, M., Sandrock, C., Leiber, F., Mathys, A., Gold, M., Zurbrugg, C., Gangnat, I.D.M., Kreuzer, M., Terranova, M., 2021. Black soldier fly larvae meal and fat can completely replace soybean cake and oil in diets for laying hens. Poult. Sci. 100, 101034. https://doi.org/10.1016/j.psj.2021.101034.

Heuel, M., Kreuzer, M., Sandrock, C., Leiber, F., Mathys, A., Guggenbühl, B., Gangnat, I.D.M., Terranova, M., 2022. Feeding value of black soldier fly larvae compared to soybean in methionine- and lysine-deficient laying hen diets. J. Insects Food Feed 8, 989−999. https://doi.org/10.3920/JIFF2021.0178.

Irawan, A., Muchiri, R.N., Parker, N.B., van Breemen, R.B., Ates, S., Bionaz, M. 2024. Cannabinoid residuals in tissues of lambs fed spent hemp biomass and consumer's exposure assessment. Food Chem. Toxicol. 191, 114848. https://doi.org/10.1016/j.fct.2024.114848.

Irawan, A., Buffington, H., Ates, S., Bionaz, M., 2025a. Use of industrial hemp byproducts in ruminants: a review of the nutritional profile, animal response, constraints, and global

regulatory environment. J. Cannabis Res. 7, 25. https://doi.org/10.1186/s42238-025-00279-7.

Irawan, A., Nosal, D.G., Muchiri, R.N., van Breemen, R.B., Ates, S., Cruickshank, J., Ranches, J., Estill, C.T., Thibodeau, A., Bionaz, M. 2025b. Cannabinoid distribution and clearance in feeding spent hemp biomass to dairy cows and the potential exposure to Δ9-THC by consuming milk. J. Agric. Food Chem. 73, 13934−13948. https://doi.org/10.1021/acs.jafc.5c02827.

Irawan, A., Hasan, D., Cruickshank, J., Estill, Ates, S., Trevisi, E., Ranches, J., Dolan, B., Bionaz, M. 2026. Spent hemp biomass as a feed ingredient for beef steers: effects on performance, blood parameters, behavior, cannabinoid residues, and consumer exposure levels. Animal 20, 101715. https://doi.org/10.1016/j.animal.2025.101715.

Jing, M., Zhao, S., House, J.D., 2017. Performance and tissue fatty acid profile of broiler chickens and laying hens fed hemp oil and HempOmega™. Poult. Sci. 96, 1809–1819. https://doi.org/10.3382/ps/pew476.

Kleinhenz, M.D., Magnin, D., Ensley, S.M., Griffin, J.J., Goeser, J., Lynch, E., Coetzee, J. F., 2020a. Nutrient concentrations, digestibility, and cannabinoid concentrations of industrial hemp plant components. Appl. Anim. Sci. 36, 489–494. https://doi.org/10.15232/aas.2020-02018.

Kleinhenz, M.D., Magnin, G., Lin, Z., Griffin, J., Kleinhenz, K.E., Montgomery, S., Curtis, A., Martin, M., Coetzee, J.F., 2020b. Plasma concentrations of eleven cannabinoids in cattle following oral administration of industrial hemp (*Cannabis sativa*). Sci. Rep. 10, 12753. https://doi.org/10.1038/s41598-020-69768-4.

Krebs, G.L., De Rosa, D.W., White, D.M., Blake, B.L., Dods, K.C., May, C.D., Tai, Z.X., Clayton, E.H., Lynch, E.E., 2021. Intake, nutrient digestibility, rumen parameters, growth rate, carcase characteristics and cannabinoid residues of sheep fed pelleted rations

containing hemp (*Cannabis sativa* L.) stubble. Transl. Anim. Sci. 5, 213. https://doi.org/10.1093/tas/txab213.

Lanzoni, D., Skřivan, M., Englmaierová, M., Petrosillo, E., Marchetti, L., Skřivanová, V., Bontempo, V., Rebucci, R., Baldi, A., Giromini, C., 2025. Effects of dietary hemp coproduct inclusion on laying hens performances and on egg nutritional and functional profile. Ital. J. Anim. Sci. 24, 248–265. https://doi.org/10.1080/1828051X.2024.2449445.

Laudadio, V., Ceci, E., Lastella, N.M.B., Introna, M., Tufarelli, V., 2014. Low-fiber alfalfa (*Medicago sativa* L.) meal in the laying hen diet: Effects on productive traits and egg quality. Poult. Sci. 97, 1868–1874. https://doi.org/10.3382/ps.2013-03831.

Li, J., Carvajal, R., Bruner, L., Kaminski, N.E., 2021. The current understanding of the benefits, safety, and regulation of cannabidiol in consumer products. Food Chem. Toxicol. 157, 112600. https://doi.org/10.1016/j.fct.2021.112600.

Mertens, D.R., 2002: Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85, 1217–1240. https://doi.org/10.1093/jaoac/85.6.1217.

Neijat, M., House, J.D., 2024. Safety and efficacy of hemp-derived products in animal feeds – a narrative review. Can. J. Anim. Sci. 104, 390–410. https://doi.org/10.1139/cjas-2023-0133.

Öztürk, E., Darmawan, A., Özlü, S., Abacı, S.H., 2024. Effects of dietary local hemp seed meal as soybean meal alternative on productive performance, egg quality and yolk fatty acid composition of laying hens, Arch. Anim. Nutr. 78, 177–190. https://doi.org/10.1080/1745039X.2024.2373485.

Parker, N.B., Hermes, J., Irawan, A., Pearce, C., Ferschweiler, D., Ates, S. Bionaz, M., 2026. Spent hemp biomass in broiler diets: Effects on performance, meat quality, expression of genes in internal adipose tissue and corticosterone in feathers. Poult. Sci. 105, 107692. https://doi.org/10.1016/j.psj.2026.107692.

Journal Pre-proof

Russo, E.B., Marcu, J., 2017. Chapter 3: Cannabis pharmacology: The usual suspects and a few promising leads. Adv. Pharmacol. 80, 67–134. https://doi.org/10.1016/bs.apha.2017.03.004.

Shariatmadari, F., 2023. Emergence of hemp as feed for poultry, World's Poult. Sci. J. 79, 769–782. https://doi.org/10.1080/00439339.2023.2234871.

Smith, D.J., Serum, E.M., Winders, T.M., Neville, B., Herges, G.R., Dahlen, C.R., Swanson, K.C., 2023. Excretion and residue depletion of cannabinoids in beef cattle fed hempseed cake for 111 days. Food Additives & Contamin. A 40, 552–565, https://doi.org/10.1080/19440049.2023.2187645.

Staehli, S.N., Poetzsch, M., Kraemer, T., Steuer, A.E., 2015. Development and validation of a dynamic range-extended LC-MS/MS multi-analyte method for 11 different postmortem matrices for redistribution studies applying solvent calibration and additional (13)C isotope monitoring. Anal. Bioanal. Chem. 407, 8681–712. https://doi.org/10.1007/s00216-015-9023-5.

Stevens, S.A., Krebs, G.L., Scrivener, C.J., Noble, G.K., Blake, B.L., Dods, K.C., May, C.D., Tai, Z.X., Clayton, E.H., Lynch, E.E., Johnson, K.N., 2022. Nutrient digestibility, rumen parameters, and (cannabinoid) residues in sheep fed a pelleted diet containing green hemp (*Cannabis sativa* L.) biomass. Translat. Anim. Sci. 6, 1–10. https://doi.org/10.1093/tas/txac141.

Storm, C., Zumwalt, M., Macherone, A., 2020. Dedicated cannabinoid potency testing in cannabis or hemp products using the Agilent 1220 Infinity II LCsSystem. Agilent Application Note on Cannabis & Hemp Testing. 10 p. https://lcms.labrulez.com/labrulez-bucket-strapi-h3hsga3/application_dedicated_cannabinoid_potency_testing_5991_9285_en_us_agilent_8306af80c2/application-dedicated-cannabinoid-potency-testing-5991-9285-en-us-agilent.pdf.

Taşkesen, H.O., Tüfekci, H., 2025. Possibilities of using hemp (*Cannabis sativa* L.) and its by-products in sheep nutrition – a review Ann. Anim. Sci. 25, 159–173. https://doi.org/10.2478/aoas-2024-0065.

Vogtmann, H., Pfirter, H.-P., Prabucki, A.L., 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Brit. Poultry Sci. 16, 531–534. https://doi.org/10.1080/00071667508416222.

Vukić Vranješ, M., Pfirter, H.-P., Wenk, C., 1994. Influence of processing treatment and type of cereal on the effect of dietary enzymes in broiler diets. Anim. Feed Sci. Technol. 46, 261–270. https://doi.org/10.1016/0377-8401(94)90144-9.

Wakshlag, J.J., Schwark, W.W., Deabold, K.A., Talsma, B.N., Cital, S., Lyubimov, A., Iqbal, A., Zakharov, A., 2020. Pharmacokinetics of cannabidiol, cannabidiolic acid, Δ9-tetrahydrocannabinol, tetrahydrocannabinolic acid and related metabolites in canine serum after dosing with three oral forms of hemp extract. Front. Vet. Sci. 7, 505. https://doi.org/10.3389/fvets.2020.00505.

Wang, S., Kreuzer, M., Braun, U., Schwarm, A., 2017. Effect of unconventional oilseeds (safflower, poppy, hemp, camelina) on in vitro ruminal methane production and fermentation. J. Sci. Food Agric. 97, 3864–3870. https://doi.org/10.1002/jsfa.8260.

Wang, K., Arshad, U., Niu, M., 2025. Enhancing quality of dairy cattle research through adequate power analysis. J. Dairy Sci. 108, 8981–9003. https://doi.org/10.3168/jds.2024-25793.

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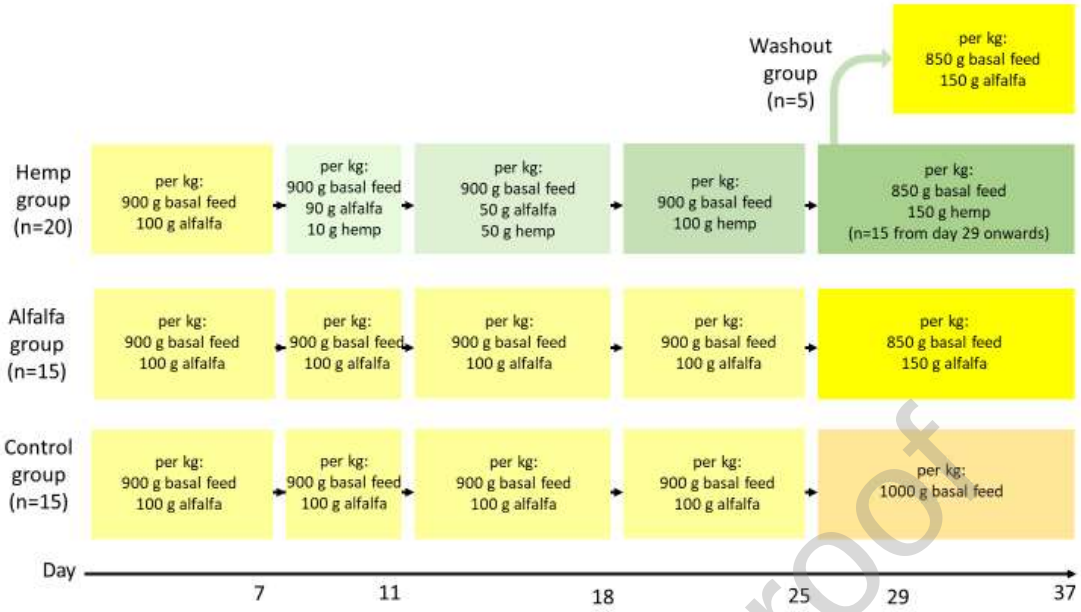


Fig. 1. Illustration of the experimental schedule.

Table 1. Composition of the three final experimental diets with 0 (Control) or 150 g/kg of alfalfa and hemp flower residue, respectively.

Items	Control	Alfalfa	Hemp
Ingredients (in g/kg as fed)			
Maize	453	385	385
Alfalfa	–	150	–
Hemp flower residue	–	–	150
Soybean meal	102	86.7	86.7
Wheat	100	85.0	85.0
Calcium carbonate	90.7	77.1	77.1
Mill by-products mix	50.0	42.5	42.5
Sunflower meal	50.0	42.5	42.5
Wheat bran	43.5	37.0	37.0
Rapeseed cake	40.0	34.0	34.0
Maize gluten	17.0	14.5	14.5
Pig fat	13.0	11.1	11.1
Soybean oil	12.1	10.3	10.3
Oyster shells	5.00	4.25	4.25
Wheat husks	4.11	3.49	3.49
Sodium bicarbonate	3.25	2.76	2.76
Monocalcium phosphate	3.05	2.59	2.59
Sodium chloride	1.80	1.53	1.53

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Bentonite	1.50	1.28	1.28
Charcoal	1.11	0.94	0.94
Premix[a]	9.50	8.08	8.08
Celite	1.60	1.60	1.60
Nutrients as analyzed (in g/kg DM unless indicated otherwise)			
Dry matter	891	900 (951)[b]	898 (929)
Organic matter	862	854 (893)	812 (711)
Crude protein	172	169 (141)	175 (236)
Ether extract	48	44 (21)	50 (35)
Neutral detergent fiber (aNDFom)	239	276 (473)	220 (263)
Gross energy (MJ/kg DM)	16.9	16.6 (17.8)	15.9 (14.0)

[a] Provided per kg of diet: 10’000 IU vitamin A, 3000 IU vitamin D_3, 50 IU vitamin E, 10 mg Cu, 88 mg Zn, 0.28 mg Se, 90 mg Fe, 123 mg Mn, 0.50 mg I, 0.09 mg Mb.

[b] Values in brackets are the analyzed values for pure alfalfa and hemp flower residue, respectively.

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Table 2. Least square means and SEM of the means of performance and egg quality measured in the hens during the last experimental week fed with the highest inclusion level [0 (Control) or 150 g/kg of alfalfa and hemp flower residue, respectively].

Diet	Control	Alfalfa	Hemp	SEM	*P*-value
Hens (n)	14	14	15		
Feed consumption (g/d per hen)	132	138	130	4.3	0.415
Intake of metabolizable energy (MJ/day per hen)[c]	1.52[a]	1.35[a,b]	1.22[b]	0.048	<0.001
BW at slaughter (kg)	1.71	1.68	1.66	0.048	0.668
Laying performance (eggs/day)	95.5	97.3	94.2	3.42	0.738
Egg weight (g)	67.5	66.6	65.1	1.07	0.273
Egg mass (g/day per hen)	64.5	64.9	61.2	1.71	0.245
Feed efficiency (g of feed/g of egg)	2.06	2.13	2.14	0.064	0.661
Metabolizability of the diet[c]					
Energy	0.76[a]	0.66[b]	0.66[b]	0.008	<0.001
Nitrogen	0.53[a]	0.45[b]	0.42[b]	0.015	<0.001
Egg quality	33.3	36.0	35.0	2.81	0.797
Shell strength (N)	33.3	36.0	35.0	2.81	0.797
Egg composition (g/kg)					
Yolk	281	294	297	5.6	0.110
Albumen	626	614	610	6.4	0.173
Shell	93	92	93	1.8	0.838
Yolk color					
Lightness (L*)	55.5[a]	53.7[ab]	52.5[b]	0.52	<0.001
Redness (a*)	0.77[b]	1.44[ab]	2.09[a]	0.209	<0.001
Yellowness (b*)	40.1	41.3	41.4	0.62	0.234
Haugh units	89.7	89.1	85.8	1.39	0.099

[a,b] Least square means within a row with no common superscript are significantly different ($P < 0.05$).

[c] As measured by excreta analysis.

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Table 3. Analyzed concentrations of cannabinoids in the hemp flower residue, the diet containing 150 g hemp flower residue/kg as well as blood plasma and breast muscle tissue of the hens fed this diet either throughout to slaughter (n = 15) or with 9 days of washout with alfalfa (n = 5) (arithmetic means ± SD of samples with detected cannabinoid concentrations).

Compound	Cannabidiol (CBD)			Δ9-Tetrahydrocannabinol (THC)		
Compound	Decarboxylated	Acidic (CBDA)	Total[a]	Decarboxylated	Acidic (THCA)	Total[b]
Hemp flower residue (mg/kg as fed)	6.25	132.0	122.0	1.74	5.05	6.16
Hemp diet (mg/kg as fed, calculated)	0.94	19.8	18.3	0.26	0.76	0.92
Plasma (μg/L)						
Hemp group[c]	36.3 ± 28.0	3,357 ± 1,358	3,393 ± 1,376	16.7 ± 13.0	385 ± 254	402 ± 255
Washout group	1.9 ± 2.2***[d]	133 ± 85***	135 ± 87***	0.4 ± 0.4***	7.0 ± 6.0***	7.4 ± 6.0***
Muscle (μg/kg)						
Hemp group	4.1 ± 4.7	132 ± 107	139 ± 114	3.6 ± 2.9	41 ± 31	47 ± 32
Washout group	n.d.***	8.2 ± 8.6***[e]	8.2 ± 8.6***	0.2 ± 0.5***[f]	n.d.***	0.2 ± 0.5***

n.d. = not detected.

[a] CBD + 0.877 × CBDA (Stevens et al., 2022).

[b] THC + 0.877 × THCA (Stevens et al., 2022).

[c] Plasma data from one hen were clearly incorrect (reason unknown) and therefore removed from statistical analysis.

[d] ***Difference between Hemp group and Washout group (after 9 d of washout) significant at $P < 0.001$ after log transformation of the data.

[e] CBDA detected in four of five hens.

[f] THC detected in two of five hens.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- The residue of cannabidiol extraction from hemp flowers was tested in laying hens
- Its feeding value was like that of alfalfa, and egg yolk coloring was superior.
- Low, but detectable levels of cannabinoids were determined in blood and muscle.
- Nine days of washout reduced cannabinoid concentration in blood and muscle.
- Hemp flower residue could be a future feed; this at least for broilers and rearing hens.

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