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Effects of Insect-Derived Feed Additives and Stocking Density in Broilers: Growth Performance, Footpad Dermatitis, Carcass Yield and Gut Health

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Simple Summary

Raising more broilers per square meter lowers the cost of each bird but can harm bird welfare and performance. Insect-derived products, such as chitosan (obtained from chitin) and black soldier fly oil, are being studied as sustainable feed ingredients that may support growth and gut health. We tested chitosan (0.05% and 0.10%) and black soldier fly oil (replacing 15% or 30% of soybean oil) in broilers kept at two stocking densities (low, 25.8 Kg/m^2; high, 38.2 Kg/m^2). Replacing 30% of the soybean oil with black soldier fly oil increased final body weight by about 8% compared with the control diet. Thigh yield was higher with the higher chitosan level and with both oil levels, and the highest oil level also increased beneficial lactic acid bacteria in the cecum. Higher stocking density increased footpad lesions regardless of diet, so neither additive offset the welfare cost of crowding. Black soldier fly oil appears to be a promising fat source for broilers, but it cannot replace good stocking management.

Abstract

High stocking density impairs broiler welfare and performance, and insect-derived feed additives may help to counteract these effects. This study evaluated dietary chitosan or black soldier fly oil (BSFO) at two stocking densities. A total of 750 one-day-old Indian River broilers were assigned to a 5 × 2 factorial arrangement of five diets (control, T1; chitosan at 0.05%, T2, or 0.10%, T3; BSFO replacing 15%, T4, or 30%, T5, of dietary soybean oil) and two stocking densities (low, 25.8 Kg/m^2; high, 38.2 Kg/m^2), with five pens per combination, for 35 d. Birds fed 30% BSFO had higher final body weight (2232 vs. 2064 g; $p = 0.011$) and weight gain than the control, without significant changes in feed conversion ratio. Relative thigh weight was higher in T3, T4 and T5 than in T1 and T2 ($p = 0.026$). Cecal *Lactobacillus* counts were higher in T5 than in T1 (6.51 vs. 5.51 $\log_{10}$ CFU/g; $p = 0.014$). Ileal villus width and muscle thickness differed among diets, but villus height, crypt depth and their ratio did not. High density reduced third-week body weight and feed intake and increased footpad dermatitis scores (1.00 vs. 0.56; $p < 0.001$), with no diet × density interaction for footpad dermatitis. In conclusion, BSFO, particularly at 30% replacement, improved growth and cecal *Lactobacillus* counts, whereas chitosan had limited effects at the doses tested. Neither additive alleviated the welfare cost of high stocking density.

Keywords: black soldier fly; chitosan; cecal microbial populations; lauric acid

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Academic Editors: Dimitrios Galamatis, Ilias Giannenas and Vasilios Tsiouris

Received: 28 October 2026
Revised: 19 November 2026
Accepted: 23 November 2026
Published: 26 November 2026

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1. Introduction

As global demand for poultry meat rises, broiler producers must increase output per unit of space and feed while keeping production sustainable and protecting bird welfare. To use house space efficiently and lower production costs, broilers are often reared at high stocking density, one of the main constraints on modern broiler production. Restricted space alters natural behavior, limits preening, reduces air exchange at bird level, and hinders the dissipation of body heat [1]. High stocking density has also been shown to impair growth performance, breast meat quality, and intestinal barrier function [2].

Feed is a major economic burden, accounting for up to 75% of total poultry production costs [3–5]. Insect meal, mainly from black soldier fly larvae (BSFL), is one of the few alternatives that can partially replace soybean meal in commercial broiler diets, and inclusion levels of up to 10–15% generally support growth performance comparable to conventional diets [6–8]. BSFL convert organic waste into protein, lipids, and chitin [9], thereby supporting waste recycling without competing directly with human food. Their protein content ranges from approximately 32% to 53%, depending on larval stage and rearing substrate [5], and their amino acid profile is comparable to that of soybean meal [10].

Feeding trials support the use of BSFL meal at moderate inclusion levels. Full-fat insect meals (*Hermetia illucens* and *Tenebrio molitor*) at 2% and 4% were incorporated without adverse effects on broiler growth, health, or carcass quality [11]. Replacing 15% of dietary soybean meal with BSFL meal is not expected to impair growth performance [12], and inclusion below 20% generally had no adverse effects on growth, blood indicators, or meat quality [10]. However, complete replacement of soybean meal is not advisable because it may reduce nutrient digestibility, an effect partly linked to the chitin content of the larvae [13], and it reduced growth performance and altered organ morphology up to 49 days of age [14].

Chitosan is a deacetylated biopolymer derived from chitin, including insect chitin, with reported antimicrobial, antioxidant, and immunomodulatory properties. In broilers, dietary chitosan at 2 g/kg improved feed conversion ratio (FCR) and the villus height-to-crypt depth ratio, increased intestinal *Bacillus* spp. counts, and reduced *Escherichia coli* counts [15]. Chitosan oligosaccharide improved body weight gain and intestinal morphology, although responses varied with inclusion level [16], and chitosan nanoparticles at 0.4 g/kg improved growth performance, carcass characteristics, and meat quality [17].

BSF larvae fat is a second insect-derived product of interest. It is rich in medium-chain fatty acids, particularly lauric acid, which have antimicrobial activity, and it can replace part or all of the soybean oil in broiler diets without impairing growth or carcass traits [18,19].

Evidence on insect-derived feed additives under environmental stress in broilers remains limited and is dominated by single-stressor designs. Fermented defatted mealworm larvae meal was tested at two stocking densities [20], BSFL protein and oil were evaluated under cyclic heat stress, where they gave no notable growth benefit [21], and a chitosan-based additive was tested at a single high density [22]. No study has compared chitosan and BSFO, two insect-derived additives with different modes of action, in a factorial design across stocking densities while recording growth, footpad dermatitis, carcass yield and gut microbial and histological indicators. The novelty of this study is therefore the simultaneous evaluation of both additives at two densities, which tests whether either can offset the welfare cost of crowding. We hypothesized that chitosan or BSFO would improve growth, gut health, and welfare indicators, particularly at high stocking density. Therefore, the study aimed to evaluate the effects of dietary chitosan or BSFO and stocking density on growth performance, footpad dermatitis, carcass traits, digestive tract length, ileal histomorphology, and litter and cecal microbial populations of broilers.

2. Materials and Methods

2.1. Ethical Statement

The experiment was conducted between October and November 2025. All procedures involving broiler chickens were approved by the Institutional Animal Care and Use Committee of Alexandria University, Egypt (approval number AU-082303262134). Efforts were made to minimize animal suffering throughout the study.

2.2. Birds, Housing, Experimental Design and Diets

A total of 750 one-day-old Indian River broiler chicks were obtained from a commercial hatchery and housed in a semi-closed house in floor pens (1 × 1 m) bedded with wood shavings. The birds were randomly allocated to 50 experimental pens, with 25 pens assigned to the low-density treatment (12 birds per pen) and 25 pens assigned to the high-density treatment (18 birds per pen). Thus, the low- and high-density treatments comprised 300 and 450 birds, respectively. Birds were randomly assigned to five dietary treatments (T1, control; T2 and T3, chitosan supplementation at 0.05% and 0.10%, respectively; and T4 and T5, replacement of 15% and 30% of dietary soybean oil with black soldier fly oil [BSFO], respectively) and two stocking-density levels (low and high, corresponding to 25.8 and 38.2 kg/m^2, respectively). Each of the 10 treatment combinations consisted of five replicate pens, with each pen serving as the experimental unit. Accordingly, the experiment followed a completely randomized design with a 5 × 2 factorial arrangement. All chicks had been immunized with the vaccines against Marek's disease, Newcastle disease, and infectious bursal disease at one day old. The birds were reimmunized against Newcastle disease on the 12th and 24th day of age, and against Gumboro disease on the 14th and 23rd day of age. House temperature was maintained at approximately 32–33 °C during the first week and then gradually decreased, averaging 27.4 ± 0.58 °C (mean ± SEM) over the whole experimental period, while relative humidity averaged 54.5 ± 1.15%. A standard lighting program and vaccination schedule, as recommended in the broiler management guide, were applied. Feed and water were provided ad libitum. Birds received starter, grower, and finisher diets formulated to meet their nutrient requirements according to the management guide of the company (Table 1).

Table 1. Ingredient and chemical composition of experimental diets.

	Control Diets			**15% BSFO Diets**			**30% BSFO Diets**		
Ingredient (kg/1000 kg) or nutrient	Starter (1–10 d)	Grower (11–25 d)	Finisher (26–35 d)	Starter (1–10 d)	Grower (11–25 d)	Finisher (26–35 d)	Starter (1–10 d)	Grower (11–25 d)	Finisher (26–35 d)
Yellow corn	549	612	675	549	612	675	549	612	675
Soybean meal (46%)	360	285	210	360	285	210	360	285	210
Corn gluten meal (60%)	30	45	60	30	45	60	30	45	60
Soybean oil	20	20	20	17	17	17	14	14	14
BSF oil	0	0	0	3	3	3	6	6	6
Limestone	13.6	12.7	12.2	13.6	12.7	12.2	13.6	12.7	12.2
Monocalcium phosphate	12	10	8	12	10	8	12	10	8
Sodium bicarbonate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Salt (NaCl)	2.6	2.2	1.9	2.6	2.2	1.9	2.6	2.2	1.9
Vitamin-mineral premix [1]	3	3	3	3	3	3	3	3	3
Choline chloride	1	1	1	1	1	1	1	1	1
DL-Methionine	2.2	1.7	1.2	2.2	1.7	1.2	2.2	1.7	1.2

Table 1. *Cont.*

	Control Diets			15% BSFO Diets			30% BSFO Diets		
L-Lysine	1.5	2.3	2.6	1.5	2.3	2.6	1.5	2.3	2.6
Phytase (5000)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Energy enzyme complex	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Biological toxin binder	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Silicate toxin binder	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Anticoccidial additive	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Anti-Clostridial additive	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Chemical composition									
Crude protein (%)	23	21	19	23	21	19	23	21	19
Metabolizable energy (kcal/kg)	3000	3100	3200	3000	3100	3200	3000	3100	3200
Ether extract (%)	4.6	4.8	5.0	4.6	4.8	5.0	4.6	4.8	5.0
Fiber (%)	2.2	2.1	2.1	2.2	2.1	2.1	2.2	2.1	2.1
Calcium (%)	1.0	0.92	0.85	1.0	0.92	0.85	1.0	0.92	0.85
Available phosphorus (%)	0.5	0.46	0.43	0.5	0.46	0.43	0.5	0.46	0.43
Lysine (%)	1.38	1.24	1.0	1.38	1.24	1.0	1.38	1.24	1.0
Methionine + cystine (%)	1.0	0.95	0.86	1.0	0.95	0.86	1.0	0.95	0.86

BSFO = black soldier fly oil. [1] Each 3 kg of premix contains: vitamins: A, 15,000,000 IU; D_3, 5,000,000 IU; E, 10,000 mg; K_3, 2000 mg; B_1, 1000 mg; B_2, 5000 mg; B_6, 1500 mg; B_{12}, 10 mg; biotin, 50 mg; pantothenic acid, 10,000 mg; nicotinic acid, 30,000 mg; folic acid, 1000 mg; minerals: Mn, 60,000 mg; Zn, 50,000 mg; Fe, 30,000 mg; Cu, 10,000 mg; I, 1000 mg; Se, 100 mg; and Co, 100 mg. The low inclusion levels of chitosan (0.05% and 0.10%) did not materially alter the overall composition of the basal control diet. Chitosan was obtained from EGYMAG Biotechnology Company, El-Amal Street, Banha 2, Al-Qalyubia Governorate 13511, Egypt.

2.3. Growth Performance and Footpad Dermatitis

Body weight was measured weekly with an electronic balance. Feed intake was recorded per replicate from d 0 to 35 and calculated weekly. Mortality was recorded daily, and feed intake was adjusted for mortality as it occurred. Body weight gain per pen was calculated as the difference between pen weight at d 35 and at d 0. FCR was calculated for each replicate as feed intake divided by body weight gain. At 35 d, 10 birds per treatment combination (two birds per replicate pen) were randomly selected for footpad dermatitis (FPD) scoring. Footpads were scored 0 (no lesions), 1 (mild lesions) or 2 (severe lesions) [23]. The score of each bird was taken as the higher of the scores for its two feet, and the pen mean of the two scored birds was used for analysis.

2.4. Carcass Characteristics

At the end of the experiment, 10 birds per treatment combination (sex-balanced) were randomly selected and weighed. After an 8 h feed withdrawal, birds were slaughtered by jugular vein bleeding at a commercial broiler slaughterhouse. After complete bleeding, the birds were defeathered, and the feet, head, and wingtips were removed. Birds were then eviscerated to determine dressed carcass weight. The drumstick, thigh, wing, and breast muscles (pectoralis major and minor) from the right side and the abdominal fat

were dissected and weighed. The liver, heart, and gizzard were also excised and weighed individually. All carcass parts were expressed as a percentage of live body weight.

2.5. Digestive Tract Length

At 35 d of age, two birds per pen were slaughtered. The total gut (proventriculus to cloaca), the intestine, and the ceca were measured with a measuring tape without stretching, and lengths were expressed in cm per bird.

2.6. Litter and Cecal Microbial Counts

At 35 d of age, litter samples were collected from each pen (two samples/pen) to assess microbial counts. Cecal contents were collected from the slaughtered birds and immediately kept on ice until they were transported to the laboratory for microbial analysis. Total bacterial counts were determined on Plate Count Agar (Merck, Germany) after incubation at 30 °C for 48 h. Coliforms were counted on Violet Red Bile Agar (Biolife) after incubation at 37 °C for 24 h. *Escherichia coli* were counted on Eosin Methylene Blue (EMB) agar (Merck) after incubation at 37 °C for 24 h. *Clostridium perfringens* were cultured on Reinforced Clostridial Agar (Oxoid CM0151) and incubated anaerobically at 37 °C for 48 h. Each sample was analyzed in triplicate, and results were expressed as $\log_{10}$ colony-forming units (CFU) per gram of sample.

2.7. Ileal Histomorphology

Ileal samples were collected immediately after slaughter (n = 5 per combination of dietary treatment by density). A 2 cm segment of distal ileum, taken 10 cm proximal to the ileocecal junction, was excised, flushed with phosphate-buffered saline (PBS, pH 7.0), fixed for 45 min in Clarke's fixative (75% ethanol and 25% glacial acetic acid; Merck, Darmstadt, Germany), and stored in 50% ethanol until processing. Cross-sections (5 µm thick) were cut with a microtome, mounted on glass slides, and stained with hematoxylin and eosin. Villus height, villus width, crypt depth, and muscle thickness were measured in µm using a light microscope (Olympus CX31, Olympus, Tokyo, Japan). For each intestinal sample, three intact villi were randomly selected for measurement. Villi were considered suitable for measurement when they exhibited a clearly identifiable tip and base, an intact and continuous epithelial lining, and no apparent tissue damage, folding, or sectioning artifacts. Villus height was measured from the villus tip to the villus–crypt junction, and crypt depth from the crypt base to the villus–crypt junction. The villus height-to-crypt depth ratio (V/C) was calculated for each bird by dividing mean villus height by mean crypt depth from the same section. Three measurements per variable were taken per sample, and the mean was used for analysis.

2.8. Statistical Analysis

Data were subjected to a two-way ANOVA using JMP software version 13.0 (SAS Institute Inc., Cary, NC, USA) with dietary treatment and stocking density as fixed effects. Prior to statistical analysis, the data were examined for normality using the Shapiro–Wilk test, while the homogeneity of variances was tested using Levene's test. The pen was the experimental unit (n = 5 per diet × density combination). Body weight was analyzed separately at each sampling age to evaluate treatment effects at specific developmental stages. Measurements on individual birds (carcass traits, gut length, ileal histomorphology, litter and cecal contents) were averaged within each pen before analysis to avoid pseudoreplication. The data were analyzed as a 5 × 2 factorial arrangement (5 dietary treatments and 2 stocking densities) following the statistical model:

$$Y_{ijk} = \mu + D_i + S_j + (D \times S)_{ij} + e_{ijk},$$

where

Y_{ijk} = observed response;
μ = overall mean;
D_i = fixed effect of dietary treatment;
S_j = fixed effect of stocking density;
$(D \times S)_{ij}$ = interaction between dietary treatment and stocking density;
e_{ijk} = random residual error.

When significant differences were detected, means were separated using Tukey's multiple-range test. Statistical significance was declared at $p < 0.05$.

3. Results

3.1. Growth Performance and Footpad Dermatitis

Dietary treatment significantly affected body weight (BWT) at the third and fifth weeks and overall body weight gain ($p = 0.016$, 0.011 and 0.011, respectively; Table 2). At week 3, T5 (30% BSFO) had the highest BWT (1062.3 g), similar to T3 (0.10% chitosan; 1051.2 g), whereas T1 had the lowest (1007.6 g). At week 5, T5 had the highest BWT (2232.2 g), similar to T4 (2189.3 g), whereas T1 and T2 had the lowest values (2063.5 and 2070.5 g). Overall weight gain followed the same pattern (T5, 2188.3 g; T4, 2145.3 g; T1 and T2, 2019.5 and 2026.5 g). Dietary treatment did not affect feed consumption (FC), FCR or FPD score. Stocking density affected third-week BWT ($p = 0.033$), overall FC ($p = 0.026$) and FPD score ($p < 0.001$). Birds at low density had higher BWT at week 3 (1048.4 vs. 1024.3 g) and higher overall FC (3468.6 vs. 3282.8 g) than those at high density, and a lower FPD score (0.56 vs. 1.00). A diet × density interaction was detected for BWT at week 2 ($p = 0.043$) and week 3 ($p = 0.009$).

Table 2. Effects of dietary treatment and stocking density on growth performance and footpad dermatitis score of broilers.

Trait [1]	Dietary Treatment (D)					Stocking Density (S)		SEM	*p*-Value		
	T1	T2	T3	T4	T5	Low	High		D	S	D × S
BWT 1st wk	175.5	171.1	173.9	184.4	172.1	174.1	176.7	4.25	0.261	0.498	0.468
BWT 2nd wk	507.1	516.8	516.7	537.6	520.0	517.5	521.7	10.21	0.074	0.518	0.043
BWT 3rd wk	1007.6 ^{c}	1018.0 ^{bc}	1051.2 ^{ab}	1042.8 ^{abc}	1062.3 ^{a}	1048.4 ^{a}	1024.3 ^{b}	17.05	0.016	0.033	0.009
BWT 4th wk	1726.5	1693.1	1726.6	1759.4	1735.1	1743.4	1712.8	19.74	0.235	0.091	0.851
BWT 5th wk	2063.5 ^{c}	2070.5 ^{c}	2115.8 ^{bc}	2189.3 ^{ab}	2232.2 ^{a}	2147.5	2121.0	38.26	0.011	0.443	0.687
Overall FC	3454.9	3438.3	3304.6	3324.5	3356.2	3468.6 ^{a}	3282.8 ^{b}	79.40	0.596	0.026	0.639
BWT Gain	2019.5 ^{c}	2026.5 ^{c}	2071.8 ^{bc}	2145.3 ^{ab}	2188.3 ^{a}	2103.5	2077.0	38.26	0.011	0.630	0.877
FCR	1.71	1.70	1.60	1.55	1.53	1.65	1.58	0.03	0.340	0.446	0.687
Footpad dermatitis score	0.95	0.80	0.70	0.80	0.65	0.56 ^{b}	1.00 ^{a}	0.065	0.604	<0.001	0.879

[1] Growth performance recorded in grams. $^{a–c}$ Means within the same row and factor with different superscripts differ significantly ($p < 0.05$). T1 = control; T2 = 0.05% chitosan; T3 = 0.10% chitosan; T4 = 15% black soldier fly oil; T5 = 30% black soldier fly oil.

3.2. Carcass Traits

Diet, density and their interaction did not affect relative eviscerated carcass weight (Table 3). Relative weights of liver, gizzard, heart and abdominal fat were also unaffected ($p > 0.05$), although the heart tended to differ among diets ($p = 0.094$) and the D × S interaction for abdominal fat approached significance ($p = 0.061$). Diet did not affect relative breast meat or pectoralis major weight, whereas pectoralis minor tended to be lower

in the BSFO groups ($p = 0.058$), with a marginal D × S interaction ($p = 0.053$). Thigh was the only trait significantly affected by diet ($p = 0.026$): relative thigh weight was higher in T3, T4 and T5 (10.58, 10.50 and 10.65%) than in T1 and T2 (9.67 and 9.97%), an increase of about 9–10% over the control. Birds at high density had higher relative wing (7.39 vs. 7.14%; $p = 0.024$) and drumstick weights (9.33 vs. 8.91%; $p = 0.047$) than those at low density. No D × S interaction was significant.

Table 3. Effects of dietary treatment and stocking density on relative weights of carcass and giblets (% of live body weight).

Trait [1]	Dietary Treatment (D)					Stocking Density (S)		SEM	*p*-Value		
	T1	T2	T3	T4	T5	Low	High		D	S	D × S
Eviscerated carcass	71.72	71.34	71.39	71.18	71.04	71.16	71.51	0.181	0.814	0.338	0.204
Liver	2.69	2.50	2.42	2.42	2.41	2.55	2.42	0.050	0.359	0.199	0.794
Gizzard	1.25	1.19	1.17	1.25	1.20	1.24	1.18	0.022	0.611	0.142	0.204
Heart	0.50	0.50	0.45	0.47	0.45	0.48	0.47	0.008	0.094	0.500	0.974
Abdominal fat	0.87	0.82	0.81	0.83	0.85	0.83	0.84	0.031	0.972	0.867	0.061
Breast meat	23.34	22.80	23.31	22.79	22.77	23.24	22.76	0.22	0.827	0.272	0.073
Thigh	9.67 [b]	9.97 [b]	10.58 [a]	10.50 [a]	10.65 [a]	10.17	10.38	0.117	0.026	0.363	0.565
Wing	7.06	7.21	7.30	7.44	7.31	7.14 [b]	7.39 [a]	0.055	0.224	0.024	0.414
Drumstick	9.13	9.20	9.18	8.96	9.14	8.91 [b]	9.33 [a]	0.104	0.951	0.047	0.397
Pectoralis major	9.54	9.25	9.61	9.40	9.38	9.52	9.35	0.101	0.808	0.405	0.118
Pectoralis minor	2.14	2.16	2.04	2.00	1.99	2.09	2.04	0.025	0.058	0.231	0.053

[1] Carcass yield and internal organs are expressed as a relative weight of live body weight. T1 = control; T2 = 0.05% chitosan; T3 = 0.10% chitosan; T4 = 15% black soldier fly oil; T5 = 30% black soldier fly oil. [a,b] Means within a row with different superscripts differ significantly ($p < 0.05$).

3.3. Digestive Tract Length

Diet did not significantly affect gut, intestine or cecum length (Table 4). Intestine length ranged from 187.21 cm (T4) to 198.79 cm (T5) and cecum length from 16.96 cm (T1) to 19.21 cm (T3). Stocking density and the D × S interaction had no significant effect on any digestive tract length.

Table 4. Effects of dietary treatment and stocking density on digestive tract length (cm) of broilers.

Trait	Dietary Treatment (D)					Stocking Density (S)		SEM	*p*-Value		
	T1	T2	T3	T4	T5	Low	High		D	S	D × S
Gut length, cm	233.68	218.43	225.86	216.00	225.21	225.91	221.76	2.16	0.074	0.320	0.329
Intestine length, cm	193.71	187.86	188.64	187.21	198.79	193.17	189.31	2.23	0.368	0.364	0.290
Cecum length, cm	16.96	17.93	19.21	18.43	18.36	18.49	17.87	0.35	0.350	0.378	0.427

T1 = control; T2 = 0.05% chitosan; T3 = 0.10% chitosan; T4 = 15% black soldier fly oil; T5 = 30% black soldier fly oil.

3.4. Litter and Cecal Microbial Counts

Diet and stocking density did not significantly affect total bacterial, *E. coli* or *Lactobacillus* counts in litter at 35 d (Table 5). Total bacterial counts ranged from 5.68 to 5.83 $\log_{10}$ CFU/g among diets and were 5.75 and 5.73 $\log_{10}$ CFU/g at low and high density; *E. coli* counts ranged from 1.00 to 1.28 $\log_{10}$ CFU/g; and *Lactobacillus* counts from 6.65 to 6.80 $\log_{10}$ CFU/g. No D × S interaction was significant. *C. perfringens* and *Salmonella* spp. were not detected in any litter sample.

Table 5. Effects of dietary treatment and stocking density on litter microbial counts (log_{10} CFU/g) at 35 d.

Trait	Dietary Treatment (D)					Stocking Density (S)		SEM	*p*-Value		
	T1	T2	T3	T4	T5	Low	High		D	S	D × S
Total bacterial count	5.69	5.79	5.68	5.83	5.70	5.75	5.73	0.06	0.275	0.705	0.240
E. coli	1.23	1.22	1.00	1.00	1.28	1.20	1.09	0.12	0.263	0.276	0.629
Lactobacillus spp.	6.65	6.80	6.80	6.67	6.77	6.71	6.76	0.05	0.135	0.269	0.984
Clostridium perfringens	ND	ND	ND	ND	ND	ND	ND	-	-	-	-
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	-	-	-	-

Clostridium perfringens and *Salmonella* spp. were not detected in any of the samples. T1 = control; T2 = 0.05% chitosan; T3 = 0.10% chitosan; T4 = 15% black soldier fly oil; T5 = 30% black soldier fly oil. ND = not detected.

In the cecum (Table 6), diet significantly affected *Lactobacillus* counts ($p = 0.014$): T5 had the highest count (6.51 log_{10} CFU/g), significantly higher than T1 (5.51 log_{10} CFU/g), whereas T2, T3 and T4 (6.03, 5.81 and 5.79 log_{10} CFU/g) were intermediate. Total bacterial and *E. coli* counts were unaffected by diet, and no cecal count was affected by density or the D × S interaction. *C. perfringens* and *Salmonella* spp. were not detected in cecal samples.

Table 6. Effects of dietary treatment and stocking density on cecal microbial counts (log_{10} CFU/g) at 35 d.

Trait	Dietary Treatment (D)					Stocking Density (S)		SEM	*p*-Value		
	T1	T2	T3	T4	T5	Low	High		D	S	D × S
Total bacterial count	4.77	5.06	4.92	4.76	5.28	4.97	4.94	0.21	0.359	0.883	0.657
E. coli	1.44	1.16	1.15	1.21	1.23	1.29	1.18	0.11	0.388	0.269	0.686
Lactobacillus spp.	5.51 ^{b}	6.03 ^{ab}	5.81 ^{ab}	5.79 ^{ab}	6.51 ^{a}	5.97	5.90	0.20	0.014	0.663	0.558
Clostridium perfringens	ND	ND	ND	ND	ND	ND	ND	-	-	-	-
Salmonella spp.	ND	ND	ND	ND	ND	ND	ND	-	-	-	-

^{a,b} Means within a row with different superscripts differ significantly ($p < 0.05$). *Clostridium perfringens* and *Salmonella* spp. were not detected in any of the samples. T1 = control; T2 = 0.05% chitosan; T3 = 0.10% chitosan; T4 = 15% black soldier fly oil; T5 = 30% black soldier fly oil. ND = not detected.

3.5. Ileal Histomorphology

Diet significantly affected ileal muscle thickness ($p = 0.006$) and villus width ($p = 0.043$; Table 7). Muscle thickness was greatest in T4 (271.33 µm), higher than in T1 (230.01 µm) and T2 (225.75 µm), whereas T3 (259.48 µm) and T5 (241.17 µm) did not differ from either group. Villus width was greatest in T5 (181.59 µm), significantly exceeding T1 (129.44 µm), whereas T2, T3 and T4 were intermediate. Crypt depth tended to be affected by diet ($p = 0.070$; 366.51 µm in T5 to 427.51 µm in T2), but villus height and V/C were not. Stocking density had no significant effect, but a diet × density interaction was detected for villus width ($p = 0.036$). The T5 diet at high density showed wider villi (225.0 µm) when compared with most other combinations. No other interaction was significant.

Table 7. Effects of dietary treatment and stocking density on ileal histomorphology of broilers.

Trait	Dietary Treatment (D)					Stocking Density (S)		SEM	*p*-Value		
	T1	T2	T3	T4	T5	Low	High		D	S	D × S
Muscle thickness, µm	230.01 ^{b}	225.75 ^{b}	259.48 ^{ab}	271.33 ^{a}	241.17 ^{ab}	242.19	248.91	4.64	0.006	0.455	0.439
Crypt depth, µm	387.60	427.51	419.60	418.08	366.51	409.30	398.46	7.87	0.070	0.488	0.449
Villus length, µm	1269.44	1242.25	1211.77	1248.65	1170.27	1218.97	1237.99	17.70	0.446	0.594	0.599
Villus width, µm	129.44 ^{b}	140.86 ^{ab}	139.54 ^{ab}	134.61 ^{ab}	181.59 ^{a}	137.60	152.80	6.11	0.043	0.197	0.036
V/C	3.45	3.04	3.00	3.21	3.39	3.14	3.30	0.083	0.322	0.323	0.284

^{a,b} Means within a row with different superscripts differ significantly ($p < 0.05$). T1 = control; T2 = 0.05% chitosan; T3 = 0.10% chitosan; T4 = 15% black soldier fly oil; T5 = 30% black soldier fly oil. V/C = villus length-to-crypt depth ratio.

4. Discussion

4.1. Growth Performance and Footpad Dermatitis

Replacing soybean oil with BSFO improved final body weight and weight gain in a dose-related manner. Birds fed 30% BSFO (T5) were heaviest at weeks 3 and 5 and gained about 8% more than the control, although FCR did not differ significantly (1.53 vs. 1.71; $p = 0.340$). Feed intake was also unaffected by diet, so the growth advantage cannot be attributed simply to higher intake. It may reflect the energy contribution and fatty acid profile of BSFO, particularly its lauric acid content. Although the fatty acid composition of the BSFO was determined and is presented in Supplementary Table S1, dietary energy utilization and the fatty acid composition of the final experimental diets were not measured in this study. Published results with BSFO are variable. Somparn et al. [24] reported that replacing 50% of dietary rice bran oil with BSFL oil did not impair broiler performance, whereas replacing 75% increased FCR in the finisher and overall periods, and BSFL oil at 2% and 4% gave no notable growth benefit in Ross 308 broilers under mild cyclic heat stress [21]. Differences in oil inclusion, basal diet, fatty acid profile, and environment may explain these discrepancies. Chitosan at 0.10% (T3) increased body weight at week 3 compared with the control (1051.2 vs. 1007.6 g), but the effect did not persist to week 5, and 0.05% chitosan had no effect on growth. Chitosan nanoparticles at 0.4 g/kg improved growth and feed efficiency in broilers [17], but the effects of chitosan depend on its form, molecular characteristics, and inclusion level, so results with nanoparticles should not be extrapolated to conventional chitosan. Buyarov et al. [22] reported higher live weight with a chitosan complex at 50–100 mg/kg at 23.5 birds/m^2, which may reflect differences in product and dose.

Stocking density influenced selected growth and welfare indicators. Birds at low density had higher week-3 body weight and overall feed consumption, which may reflect better access to feeder and drinker space and less competition. However, final body weight, weight gain, and FCR did not differ significantly between densities, so the effect of density on growth was not consistent through the cycle. FPD scores were 79% higher at high density (1.00 vs. 0.56). This agrees with reports that high stocking density increases litter moisture, ammonia, and FPD [25,26], and that prolonged exposure to wet litter damages footpad integrity [27]. Litter moisture and ammonia were not measured here, so these mechanisms remain plausible explanations rather than confirmed causes. Neither diet nor the diet × density interaction affected FPD, so neither additive protected the footpads against crowding. The diet × density interactions for body weight at weeks 2 and 3 were not maintained at later ages. A significant diet × stocking density interaction was found at 14 d ($p = 0.043$); the only difference between treatment combinations was that birds fed T4 at low density were heavier than those fed T1 at low density, suggesting that the early response to the diets depended on the stocking density. At 21 d, the interaction was also significant ($p = 0.009$). The treatment T5 showed a statistically significant difference between densities (1109.1 g at low density vs. 1015.6 g at high density). For T4 (1068.3 g vs. 1017.3 g) and T3 (1053.6 g vs. 1048.8 g), the low- and high-density means share the same superscript letter and therefore do not differ significantly (Table S2). Because the interaction means that the effect of diet depends on stocking density, these results should be interpreted by comparing the diet means within each density rather than by the main effect of diet alone.

4.2. Carcass Traits

Eviscerated carcass yield was unaffected by diet, density, or their interaction and remained within a narrow range (71.04–71.72%). This agrees with studies in which BSFL fat replaced part or all of the soybean oil without altering slaughter performance [18,19]. Final body weight was higher with BSFO (Table 2), whereas relative eviscerated carcass

weight did not differ (Table 3). Thus, the additives appear to have changed bird size more than dressing proportion. The effect of chitosan on carcass yield is less consistent. Tufan and Arslan [28] reported a higher dressing percentage with chitosan oligosaccharide at 50 and 100 mg/kg, whereas the same group found no effect of 100 mg/kg on carcass ratio or breast, leg and wing ratios, although abdominal fat was lower [29]. We used chitosan rather than the oligosaccharide, at 500 and 1000 mg/kg, which is 5 to 10 times those doses. Responses to chitosan depend on molecular weight [30,31] and inclusion level [16].

The thigh was the main carcass cut responding to diet. Relative thigh weight was higher with 0.10% chitosan and both BSFO levels than with the control and 0.05% chitosan, suggesting a threshold rather than a graded response. This occurred in T3, whose final weight did not differ from the control, so it was not simply a consequence of larger birds. Tufan and Arslan [28] also reported higher thigh ratios with chitosan oligosaccharide. Possible explanations are better nutrient utilization through improved gut function, the lipid-modulating properties of chitosan, and, for BSFO, changes in muscle fatty acid composition [18]. Unchanged breast and abdominal fat suggest that the response was specific to leg muscle rather than a general shift in fat deposition. These explanations remain hypotheses because digestibility and muscle composition were not measured.

Wing and drumstick yields were higher at high density. Because cuts were expressed relative to live weight, this should not be read as a gain in muscle mass. High density reduced feed consumption and week-3 body weight and numerically reduced final weight, and absolute final body, wing, and drumstick weights decline linearly with density, mainly through reduced feed intake [32]. The higher relative weights may therefore reflect slightly smaller bodies rather than larger cuts. Unchanged breast and thigh yields agree with reports of similar carcass characteristics at moderate densities [33,34]. This does not mean that crowding was harmless, given the higher FPD scores.

4.3. Digestive Tract, Microbial Populations and Ileal Histomorphology

Neither chitosan nor BSFO changed total bacterial or *E. coli* counts in litter or cecum, and *C. perfringens* and *Salmonella* spp. were not detected. Nuengjamnong and Angkanaporn [15] reported that chitosan increased *Bacillus* spp. and lowered *E. coli* in intestinal digesta. The absence of a similar response here probably reflects the very low *E. coli* load (about 1 $\log_{10}$ CFU/g, possibly near the detection limit), which leaves little room for reduction. Differences in chitosan molecular weight, degree of deacetylation, and sampling site may also contribute. Taxon-specific shifts without changes in total load have been reported for BSF-derived products: BSFL included at up to 20% of the diet had only minor effects on cecal microbiota [35].

The higher cecal *Lactobacillus* count in T5 is consistent with the fatty acid profile of BSFO, which is rich in medium-chain fatty acids with antimicrobial activity against potential pathogens. Dabbou et al. [19] also observed a higher relative abundance of *Lactobacillus* in birds fed BSF fat, although not with every modified fat, which suggests that the response depends on fat composition. The effect appeared only in the cecum, the main site of microbial fermentation, and not in litter, which mainly reflects excreta mixed with environmental bacteria and is less responsive to diet. It was limited to the highest inclusion level, with T4 intermediate, which may indicate a dose-dependent response. Whether lactic acid production lowered cecal pH and disfavored pathogens was not examined.

Diet and density did not affect gut, intestine, or cecum length, in agreement with Dabbou et al. [19], who found that BSF fat did not influence gut morphometric indices. Chitosan oligosaccharide increased relative duodenal weight and villus surface area in broilers at d 1–14 [36], which suggests that such effects are mainly seen early in life. Unchanged villus height and V/C ratio indicate that neither chitosan nor BSFO impaired ileal structure

at either density, as also reported when BSF fat replaced soybean oil in finisher broilers [37] and turkeys [38].

Muscle thickness was greater in T4 and villus width in T5 than in the control, whereas villus height and V/C were unchanged. Higher V/C with 2 g/kg chitosan [15] and lower crypt depth with chitosan oligosaccharide at d 1–14 [36] were not reproduced here, possibly because of the chitosan form, the older age of the birds, or the sampling site. Crypt depth in T2 was numerically higher than in the control, but diet only tended to affect crypt depth ($p = 0.070$), so this should not be over-interpreted. Medium-chain fatty acids were linked to greater villus height and V/C in the duodenum and jejunum [39], whereas Dabbou et al. [19] found no change in gut morphometry. The wider villi observed in the T5 group might reflect a potential trophic effect of BSFO, particularly its lauric acid content, as indicated by the fatty acid profile presented in Supplementary Table S1. However, villus height remained unchanged, and enterocyte responses were not directly assessed. Furthermore, although the fatty acid composition of BSFO was determined, that of the final experimental diets was not analyzed. Therefore, this potential mechanism should be interpreted cautiously. Villus width was the only histological parameter showing a significant diet × stocking density interaction, with the T5 diet under high stocking density resulting in wider villi than most other treatment combinations.

Stocking density had no main effect on microbial counts, gut length, or ileal histomorphology. Studies reporting clear effects of high density mostly compared 20–22 with 14 birds/m^2: lower ileal villus height and V/C [40], lower jejunal villus height and reduced cecal microbial diversity [41], and suppressed ileal villus growth with a disturbed microbiota [42]. Sarıca et al. [20], however, reported lower ileal villus height and *Lactobacillus* content and higher *E. coli* at 18 than at 12 birds/m^2, the same densities used here, so the density range alone does not explain the difference. Bird age at sampling, breed, housing conditions, and final stocking load (kg/m^2) may be involved. Sooksridang et al. [43] likewise found no effect of 11 versus 14 birds/m^2 on jejunal morphology.

5. Conclusions

Under the conditions of this study, replacing 30% of dietary soybean oil with BSFO increased final body weight by about 8% compared with the control and raised cecal *Lactobacillus* counts, without significant changes in FCR, carcass yield, or gut length. Chitosan at 0.05% and 0.10% had limited effects, apart from a transient body weight increase at week 3 with 0.10% chitosan. Relative thigh weight was higher with 0.10% chitosan and with both BSFO levels. High stocking density (18 vs. 12 birds/m^2) reduced early body weight and feed intake and increased footpad dermatitis scores, and neither additive alleviated this effect. BSFO may therefore be a useful fat source for broilers, but it does not replace appropriate stocking-density management. Overall, the insect-derived additives acted largely independently of stocking density, with the exceptions of transient diet × density interactions for body weight at weeks 2 and 3 and for ileal villus width. BSF oil supported growth and increased cecal *Lactobacillus* counts, chitosan had modest effects at the doses tested, and crowding was the only factor affecting footpad condition.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ani16233775/s1, Table S1: Chromatographic fatty acid profile of the BSF oil; Table S2: Effects of dietary treatment (T1–T5) and stocking density (low, high) on body weight at 2 and 3 WK and villus width in broilers.

Author Contributions: Conceptualization, A.E. and A.E.-D.; methodology, A.E., A.E.-D. and H.E.; formal analysis, A.E., A.E.-D. and H.E.; investigation, A.E., A.E.-D., H.E. and M.F.; writing—original

draft preparation, A.E., H.E. and M.F.; writing—review and editing, A.E.-D. and M.F.; supervision, A.E., A.E.-D. and M.F. All authors have read and agreed to the published version of the manuscript.

Funding: The researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2026).

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee of Alexandria University, Egypt (approval number AU-082303262134, dated 26 March 2023).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank Salah El-Safty and his team for their invaluable efforts and assistance in conducting the experiment. Generative AI (Claude Sonnet 5.5, Anthropic, San Francisco, CA, USA) was used to assist with language editing, manuscript formatting, and reference formatting.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Sakr, S.A.; Abouelnaga, A.F.; Ateya, A.I.; Hashem, N.M.A.; Wahed, N.M.; Rehan, I.F.; Elnagar, A.; Zigo, F.; Siedoi, I.; Kamel, W.A.; et al. Growth Performance, Behavior, Gene Expression, Carcass Characteristics, Stress Indicators, and Economical Parameters of Broiler Chickens Raised under Three Different Stocking Densities. *Front. Vet. Sci.* **2025**, *12*, 1517142. [CrossRef] [PubMed]
2. Goo, D.; Kim, J.H.; Park, G.H.; Delos Reyes, J.B.; Kil, D.Y. Effect of Heat Stress and Stocking Density on Growth Performance, Breast Meat Quality, and Intestinal Barrier Function in Broiler Chickens. *Animals* **2019**, *9*, 107. [CrossRef] [PubMed]
3. El-Deek, A.A.; Abdel-Wareth, A.A.A.; Osman, M.; El-Shafey, M.; Khalifah, A.M.; Elkomy, A.E.; Lohakare, J. Alternative Feed Ingredients in the Finisher Diets for Sustainable Broiler Production. *Sci. Rep.* **2020**, *10*, 17743. [CrossRef] [PubMed]
4. Sumbule, E.K.; Ambula, M.K.; Osuga, I.M.; Changeh, J.G.; Mwangi, D.M.; Subramanian, S.; Salifu, D.; Alaru, P.A.O.; Githinji, M.; van Loon, J.J.A.; et al. Cost-Effectiveness of Black Soldier Fly Larvae Meal as Substitute of Fishmeal in Diets for Layer Chicks and Growers. *Sustainability* **2021**, *13*, 6074. [CrossRef]
5. Salahuddin, M.; Abdel-Wareth, A.A.A.; Hiramatsu, K.; Tomberlin, J.K.; Luza, D.; Lohakare, J. Flight toward Sustainability in Poultry Nutrition with Black Soldier Fly Larvae. *Animals* **2024**, *14*, 510. [CrossRef] [PubMed]
6. Murawska, D.; Daszkiewicz, T.; Sobotka, W.; Gesek, M.; Witkowska, D.; Matusevičius, P.; Bakula, T. Partial and Total Replacement of Soybean Meal with Full-Fat Black Soldier Fly (*Hermetia illucens* L.) Larvae Meal in Broiler Chicken Diets: Impact on Growth Performance, Carcass Quality and Meat Quality. *Animals* **2021**, *11*, 2715. [CrossRef] [PubMed]
7. Attia, Y.A.; Bovera, F.; Asiry, K.A.; Alqurashi, S.; Alrefaei, M.S. Fish and Black Soldier Fly Meals as Partial Replacements for Soybean Meal Can Affect Sustainability of Productive Performance, Blood Constituents, Gut Microbiota, and Nutrient Excretion of Broiler Chickens. *Animals* **2023**, *13*, 2759. [CrossRef] [PubMed]
8. Hayat, Z.; Riaz, T.; Attia, Y.A.; Hameed, F.; Siddiqui, F.J. Analysing the Effects of Feeding Black Soldier Fly on Broiler Growth Performance: A Meta-Analysis. *Anim. Prod. Sci.* **2024**, *64*, AN24106. Corrigendum in *Anim. Prod. Sci.* **2024**, *64*, AN24106_CO. https://doi.org/10.1071/AN24106_CO. [CrossRef]
9. Hossain, M.A.; Bhuiyan, M.J.U. Black Soldier Fly (*Hermetia illucens*): A Proteinous Substitution of Soybean and Fish Meal for Broiler and Layer Chicken: A Review. *Poult. Sci. J.* **2023**, *11*, 133–147. [CrossRef]
10. Lu, S.; Taethaisong, N.; Meethip, W.; Surakhunthod, J.; Sinpru, B.; Sroichak, T.; Archa, P.; Thongpea, S.; Paengkoum, S.; Purba, R.A.P.; et al. Nutritional Composition of Black Soldier Fly Larvae (*Hermetia illucens* L.) and Its Potential Uses as Alternative Protein Sources in Animal Diets: A Review. *Insects* **2022**, *13*, 831. [CrossRef] [PubMed]
11. Tóth, M.; Singh, Y.; Balogh, K.; Zándoki, E.; Kulcsár, S.; Kövesi, B.; Ancsin, Z.; Gregosits, B.; Mézes, M.; Kovács-Weber, M.; et al. Effect of Dietary Inclusion of Full-Fat Insect Meals (*Hermetia illucens* and *Tenebrio molitor*) for Broiler Chickens: Live Performance, Carcass Yield, Meat Quality, Blood Profiles, and Intestinal Morphometry. *Animals* **2026**, *16*, 939. [CrossRef] [PubMed]
12. Martínez Marín, A.L.; Gariglio, M.; Biasato, I.; Gasco, L.; Schiavone, A. Meta-Analysis of the Effect of Black Soldier Fly Larvae Meal in Diet on Broiler Performance and Prediction of Its Metabolisable Energy Value. *Ital. J. Anim. Sci.* **2023**, *22*, 379–387. [CrossRef]
13. Chobanova, S.; Karkelanov, N.; Mansbridge, S.C.; Whiting, I.M.; Simic, A.; Rose, S.P.; Pirgozliev, V.R. Defatted Black Soldier Fly Larvae Meal as an Alternative to Soybean Meal for Broiler Chickens. *Poultry* **2023**, *2*, 430–441. [CrossRef]

14. Facey, H.; Kithama, M.; Mohammadigheisar, M.; Huber, L.-A.; Shoveller, A.K.; Kiarie, E.G. Complete Replacement of Soybean Meal with Black Soldier Fly Larvae Meal in Feeding Program for Broiler Chickens from Placement through to 49 Days of Age Reduced Growth Performance and Altered Organs Morphology. *Poult. Sci.* **2023**, *102*, 102293. [CrossRef] [PubMed]
15. Nuengjamnong, C.; Angkanaporn, K. Efficacy of Dietary Chitosan on Growth Performance, Haematological Parameters and Gut Function in Broilers. *Ital. J. Anim. Sci.* **2018**, *17*, 428–435. [CrossRef]
16. Ayman, U.; Akter, L.; Islam, R.; Bhakta, S.; Rahman, M.A.; Islam, M.R.; Sultana, N.; Sharif, A.; Jahan, M.R.; Rahman, M.S.; et al. Dietary Chitosan Oligosaccharides Improves Health Status in Broilers for Safe Poultry Meat Production. *Ann. Agric. Sci.* **2022**, *67*, 90–98. [CrossRef]
17. Adil, S.; Aldhalmi, A.K.; Wani, M.A.; Baba, I.A.; Sheikh, I.U.; Abd El-Hack, M.E.; Aljahdali, N.; Albaqami, N.M.; Abuljadayel, D.A. Impacts of Dietary Supplementation of Chitosan Nanoparticles on Growth, Carcass Traits, Nutrient Digestibility, Blood Biochemistry, Intestinal Microbial Load, and Meat Quality of Broilers. *Transl. Anim. Sci.* **2024**, *8*, txae134. [CrossRef] [PubMed]
18. Schiavone, A.; Cullere, M.; De Marco, M.; Meneguz, M.; Biasato, I.; Bergagna, S.; Dezzutto, D.; Gai, F.; Dabbou, S.; Gasco, L.; et al. Partial or Total Replacement of Soybean Oil by Black Soldier Fly Larvae (*Hermetia illucens* L.) Fat in Broiler Diets: Effect on Growth Performances, Feed-Choice, Blood Traits, Carcass Characteristics and Meat Quality. *Ital. J. Anim. Sci.* **2017**, *16*, 93–100. [CrossRef]
19. Dabbou, S.; Lauwaerts, A.; Ferrocino, I.; Biasato, I.; Sirri, F.; Zampiga, M.; Bergagna, S.; Pagliasso, G.; Gariglio, M.; Colombino, E.; et al. Modified Black Soldier Fly Larva Fat in Broiler Diet: Effects on Performance, Carcass Traits, Blood Parameters, Histomorphological Features and Gut Microbiota. *Animals* **2021**, *11*, 1837. [CrossRef] [PubMed]
20. Sarıca, Ş.; Yavuz, M.; Sanli, E.R.; Kati, A.; Altuntaş, S.; Alataş, M.S.; Ekici, M.; Yazarel, S.; Keskintaş, A.T. Effects of Dietary Fermented Mealworm Larvae and Stocking Density on Performance, Blood Stress Indicator and Intestine Parameters of Broilers. *J. Hell. Vet. Med. Soc.* **2024**, *75*, 7887–7902. [CrossRef]
21. Veldkamp, T.; Rezaei Far, A.; Mes, J.J.; Naser El Deen, S.; van Wikselaar, P.G.; Fodor, I.; Chen, H. Effects of Inclusion Level of Black Soldier Fly Larvae Protein or Oil on Broiler Growth Performance during Heat Stress. *Poult. Sci.* **2025**, *104*, 105883. [CrossRef] [PubMed]
22. Buyarov, V.; Komolikova, I.; Buyarov, A.; Mednova, V. Efficiency of Using Chitosan Complex in Feeding Technology for Broiler Chickens under Conditions of Increased Stocking Density. *BIO Web Conf.* **2024**, *118*, 01028. [CrossRef]
23. Durmuş, M.; Kurşun, K.; Polat Açık, İ.; Tufan, M.; Kutay, H.; Benli, H.; Baylan, M.; Kutlu, H.R. Effect of Different Litter Materials on Growth Performance, the Gait Score and Footpad Dermatitis, Carcass Parameters, Meat Quality, and Microbial Load of Litter in Broiler Chickens. *Poult. Sci.* **2023**, *102*, 102763. [CrossRef] [PubMed]
24. Somparn, N.; Pootthachaya, P.; Puangsap, W.; Pintaphrom, N.; Srikha, T.; Tengjaroenkul, B.; Cherdthong, A.; Wongtangtintharn, S. Evaluation of Black Soldier Fly Larvae Oil as a Feed Ingredient for Broiler Chickens: Effects on Performance, Carcass Traits, Meat Characteristics, and Blood Parameters. *Front. Anim. Sci.* **2024**, *5*, 1496763. [CrossRef]
25. Kim, C.H.; Kim, K.H.; Chun, J.L.; Lim, S.J.; Jeon, J.H. Animal Welfare Indicators and Stress Response of Broiler Chickens Raised at Low and High Stocking Density. *J. Anim. Sci. Technol.* **2025**, *67*, 1126–1138. [CrossRef] [PubMed]
26. Youssef, I.M.I.; Beineke, A.; Rohn, K.; Kamphues, J. Effect of Alum-Amended Litter and Stocking Density on Ammonia Release and Footpad and Hock Dermatitis of Broilers. *Agric. Sci. China* **2011**, *10*, 777–785. [CrossRef]
27. Taira, K.; Nagai, T.; Obi, T.; Takase, K. Effect of Litter Moisture on the Development of Footpad Dermatitis in Broiler Chickens. *J. Vet. Med. Sci.* **2014**, *76*, 583–586. [CrossRef] [PubMed]
28. Tufan, T.; Arslan, C. Dietary Supplementation with Chitosan Oligosaccharide Affects Serum Lipids and Nutrient Digestibility in Broilers. *S. Afr. J. Anim. Sci.* **2020**, *50*, 663–671. [CrossRef]
29. Arslan, C.; Tufan, T. Effects of Chitosan Oligosaccharides and L-Carnitine Individually or Concurrent Supplementation for Diets on Growth Performance, Carcass Traits and Serum Composition of Broiler Chickens. *Rev. Med. Vet.* **2018**, *169*, 130–137.
30. Li, Q.P.; Gooneratne, S.R.; Wang, R.L.; Zhang, R.; An, L.L.; Chen, J.J.; Pan, W. Effect of Different Molecular Weight of Chitosans on Performance and Lipid Metabolism in Chicken. *Anim. Feed Sci. Technol.* **2016**, *211*, 174–180. [CrossRef]
31. Wang, R.L.; Chen, J.W.; Gooneratne, R.; He, X.P.; Huang, J.Z.; Zhao, Z.H. Effects of Varied Molecular Weight of Chitosan Oligosaccharides on Growth Performance, Carcass Trait, Meat Quality, and Fat Metabolism in Indigenous Yellow-Feathered Chickens. *J. Appl. Poult. Res.* **2022**, *31*, 100221. [CrossRef]
32. Thema, K.K.; Mnisi, C.M.; Mlambo, V. Stocking Density-Induced Changes in Growth Performance, Blood Parameters, Meat Quality Traits, and Welfare of Broiler Chickens Reared under Semi-Arid Subtropical Conditions. *PLoS ONE* **2022**, *17*, e0275811. [CrossRef] [PubMed]
33. Thomas, D.G.; Ravindran, V.; Thomas, D.V.; Camden, B.J.; Cottam, Y.H.; Morel, P.C.H.; Cook, C.J. Influence of Stocking Density on the Performance, Carcass Characteristics and Selected Welfare Indicators of Broiler Chickens. *N. Z. Vet. J.* **2004**, *52*, 76–81. [CrossRef] [PubMed]
34. Adeyemo, G.O.; Fashola, O.O.; Ademulegun, T.I. Effect of Stocking Density on the Performance, Carcass Yield and Meat Composition of Broiler Chickens. *Br. Biotechnol. J.* **2016**, *14*, 1–7. [CrossRef]

35. de Vilela Souza, J.V.; Kheravii, S.K.; Bajagayi, Y.S.; Kolakshyapati, M.; Wu, S.; Ruhnke, I. Inclusion of Black Soldier Fly Larvae in a Meat Chicken Diet Has Minor Effect on Caeca Microbiota. In *Proceedings of the Australian Poultry Science Symposium*; Roberts, J., Ed.; Poultry Research Foundation, The University of Sydney: Camden, NSW, Australia, 2021; Volume 32, p. 146. Available online: https://hdl.handle.net/1959.11/31125 (accessed on 12 October 2026).
36. Lan, R.; Wu, F.; Wang, Y.; Lin, Z.; Wang, H.; Zhang, J.; Zhao, Z. Chitosan Oligosaccharide Improves Intestinal Function by Promoting Intestinal Development, Alleviating Intestinal Inflammatory Response, and Enhancing Antioxidant Capacity in Broilers Aged d 1 to 14. *Poult. Sci.* **2024**, *103*, 103381. [CrossRef] [PubMed]
37. Schiavone, A.; Dabbou, S.; De Marco, M.; Cullere, M.; Biasato, I.; Biasibetti, E.; Capucchio, M.T.; Bergagna, S.; Dezzutto, D.; Meneguz, M.; et al. Black Soldier Fly Larva Fat Inclusion in Finisher Broiler Chicken Diet as an Alternative Fat Source. *Animal* **2018**, *12*, 2032–2039. [CrossRef] [PubMed]
38. Sypniewski, J.; Kierończyk, B.; Benzertiha, A.; Mikołajczak, Z.; Pruszyńska-Oszmałek, E.; Kołodziejski, P.; Sassek, M.; Rawski, M.; Czekała, W.; Józefiak, D. Replacement of Soybean Oil by *Hermetia illucens* Fat in Turkey Nutrition: Effect on Performance, Digestibility, Microbial Community, Immune and Physiological Status and Final Product Quality. *Br. Poult. Sci.* **2020**, *61*, 294–302. [CrossRef] [PubMed]
39. Zeitz, J.; Fennhoff, J.; Kluge, H.; Stangl, G.I.; Eder, K. Effects of Dietary Fats Rich in Lauric and Myristic Acid on Performance, Intestinal Morphology, Gut Microbes, and Meat Quality in Broilers. *Poult. Sci.* **2015**, *94*, 2404–2413. [CrossRef] [PubMed]
40. Dong, Y.; Zheng, Y.; Liu, H.; Wang, Y.; Cui, J.; Wu, Y.; Yan, L.; Miao, Z.; Han, M.; Huang, C.; et al. Effects of High Stocking Density on the Growth Performance, Intestinal Health and Bile Salts Composition of Broiler Chickens. *Front. Microbiol.* **2025**, *16*, 1542059. [CrossRef] [PubMed]
41. Zhang, H.; Zhang, Y.; Bai, D.; Zhong, J.; Hu, X.; Zhang, R.; Zhen, W.; Ito, K.; Zhang, B.; Yang, Y.; et al. Effect of Dietary Aspirin Eugenol Ester on the Growth Performance, Antioxidant Capacity, Intestinal Inflammation, and Cecal Microbiota of Broilers under High Stocking Density. *Poult. Sci.* **2024**, *103*, 103825. [CrossRef] [PubMed]
42. Li, Y.-Q.; Zhang, Y.; Bai, D.-Y.; Liu, Y.-H.; He, X.-L.; Ito, K.; Liu, K.-X.; Tan, H.-Q.; Zhen, W.-R.; Zhang, C.; et al. Effects of Dietary Chlorogenic Acid on Ileal Intestinal Morphology, Barrier Function, Immune Factors and Gut Microbiota of Broilers under High Stocking Density Stress. *Front. Physiol.* **2023**, *14*, 1169375. [CrossRef] [PubMed]
43. Sooksridang, T.; Rachatapibul, C.; Srinongkote, S.; Mukai, K.; Kikusato, M. Trehalose Supplementation Effects on Growth, Intestinal Morphology, Gut Bacteria, and Footpad Dermatitis of Broiler Chickens Reared at High Density. *J. Poult. Sci.* **2024**, *61*, jsa.2024001. [CrossRef] [PubMed]

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