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The Impact of Whole Dried Black Soldier Fly Larvae on Broiler Health and Growth During a Necrotic Enteritis Challenge

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Abstract

Increased demands for protein have led to a search for alternatives to traditional protein sources like soy and animal protein. Black Soldier Fly Larvae can be reared on many feedstocks to produce a high-quality nutrient source for livestock and pets. These insects contain biologically meaningful compounds like antimicrobial peptides, lauric acid, and chitin. This combination of compounds highlights the need to investigate BSFL as a functional ingredient in broilers. This study examined the impact of BSFL inclusion on broiler performance with and without a subclinical Necrotic Enteritis (NE) challenge over two experiments. In both experiments, diets included 0%, 2.5%, or 5.0% BSFL from 0–42 d. During Experiment 2, birds were given a live coccidiosis vaccine at hatch and challenged with *C. perfringens* at 19, 20, and 21 d. Primary variables include growth performance, lesion scores, and NE-specific mortality. A BSFL inclusion of 2.5% and 5.0% significantly improved feed conversion ratio in both experiments ($p < 0.001$); 5% BSFL inclusion also significantly improved body weight gain in both experiments ($p = 0.014$, $p = 0.023$, respectively). Overall, results indicate that BSFL is an effective and safe feed ingredient option for commercial broiler production, providing biologically relevant improvements in performance with and without disease pressure.

Keywords: Black Soldier Fly Larvae; necrotic enteritis; broiler; mortality; growth performance

1. Introduction

The demand for edible protein and novel protein sources continues to rise [1]. As antibiotic use in poultry production faces increasing regulatory and consumer restrictions, there is an urgent need for alternative feed ingredients that may improve disease outcomes.

Insect-based ingredients are growing in popularity as a source of nutrition for pet foods and livestock feeds. Primary insect ingredients such as insect meal, insect oil, whole dried insects, and live insects have demonstrated application in the diets of a variety of species [2–4]. In addition, the added benefits of lauric acid, chitin, and antimicrobial peptides from insect ingredients have improved disease outcomes and modulated the immune system in multiple species [5,6]. Although there is some evidence to support the use of insect ingredients to mitigate disease challenges in poultry, this area of research is limited and is dominated by the utilization of insect meal and insect oil.

In previous studies using BSFL ingredients in broilers, live larvae fed as enrichment did not negatively impact growth performance or overall health, but did improve welfare outcomes by serving as a natural enrichment item [7]. Additionally, defatted BSFL meal inhibited growth performance metrics when added to the diet at greater than 10%

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inclusion; however, lower inclusion rates improved or maintained performance and gut morphology [8]. BSFL Oil inclusion has primarily been tested at low levels, demonstrating improved feed conversion when provided at 0.5% of the diet [9]. Higher levels of BSFL Oil inclusion of 3.0% to 6.0% showed no changes in growth performance compared to a soy oil control group [10].

In addition to its use as an alternative protein and energy source, whole dried BSFL contains several other components that support its inclusion in commercial poultry diets such as lauric acid, chitin, and antimicrobial peptides (AMP). AMPs have demonstrated direct-killing of viruses, bacteria, fungi, and parasites through targeting of the lipopolysaccharide layer of the cell membrane [11,12]. Lauric acid, a medium-chain fatty acid commonly found in coconut oil and palm kernel oil, demonstrates increased energy availability and rapid absorption compared to many other fat sources [13]. Additionally, research shows that Lauric acid can reduce the colonization of poultry carcasses by human health pathogens like *Campylobacter* spp. [14]. Insect exoskeletons serve as an abundant source of chitin, which has demonstrated prebiotic potential in various species including poultry, where various insect sources of chitin have demonstrated improvements in growth performance and carcass quality [15]. Recently, chitin derivatives have been of interest due to their antimicrobial activity against wound-infection causing bacteria [16].

These beneficial components highlight the need for additional research to determine BSFL as a promising feed ingredient to improve growth performance and disease outcomes in broilers. In aquaculture species, whole dried BSFL has demonstrated improvements in disease outcomes when exposed to an induced enteritis model [6]. Given the previous research that shows higher inclusion rates if insect ingredients may inhibit performance in broilers, and that lower inclusion rates of 5% improve enteritis outcomes in other species, this study aims to test low levels of whole dried BSFL inclusion in broiler diets during a subclinical necrotic enteritis model. The subclinical necrotic enteritis model was chosen for this study because subclinical infections are more difficult to diagnose in the field and are better suited for feed grade interventions.

2. Materials and Methods

2.1. Ethical Statement

This study, including animal handling and associated procedures, was approved by the Southern Poultry Feed and Research Institutional Animal Care and Use Committee (Athens, GA, USA, approved on 27 January 2023 EF0223).

2.2. Allocation and Management

This study was composed of two separate experiments. In both experiments, Male Cobb 500 chicks (from the Cobb hatchery, Cleveland, GA, USA) were randomly allocated to one of three treatment groups using the randomization function in Excel: Control, 2.5% BSFL inclusion, and 5.0% BSFL inclusion. Each treatment had 10 replicate pens. In Experiment 1, there were 20 chicks per pen, for a total of 200 chicks per treatment, or 600 chicks total. Pens were 4.5 ft. by 4.5 ft for a total of 20.25 ft^2, providing approximately 1 ft^2 per bird or 0.1 m^2 per bird. In Experiment 2, there were 45 chicks per pen, for a total of 450 chicks per treatment, or 1350 chicks total. Pens were 4.5 ft. by 10 ft for a total of 45 ft^2, providing approximately 1 ft^2 per bird or 0.1 m^2 per bird. Animals were managed according to study site standard operating procedures. In general, temperatures were regularly monitored, and were managed according to the Cobb 500 management guidelines. Illumination was provided using fluorescent bulbs above the pens with lighting in accordance with the Cobb 500 management guidelines. Animals were observed a minimum of twice daily to ensure proper rearing conditions were maintained.

In Experiment 2, mortalities were necropsied to determine cause of death. This information was used to determine necrotic enteritis-specific mortality. No animals or experimental units were excluded from data analysis in this experiment. In each experiment, treatments were randomly applied to pens within the experimental house to avoid any bias or variability based on location within the experiment room. Individuals involved in data collection and analysis remained blind to treatment identification to reduce opportunities for variability and bias in study results.

2.3. Vaccination and Medication

Chicks were sprayed with a commercial coccidia vaccine (Coccivac B52, Merck Animal Health USA, Rahway, NJ, USA) at study start for both experiments using the manufacturer recommended inclusion level. No concomitant drug therapy was utilized in this study.

2.4. Diets

In both experiments, broilers were fed ad libitum in tube-type feeders for the entire 42-day study. During the first week of each trial, feed was supplemented in a tray on the floor of each pen. This study was divided into three feeding phases (starter, grower, and finisher). Diets for each phase were manufactured by Southern Poultry Research, Inc. (Athens, GA, USA) using a Davis S-20 mixer (H.C. Davis Sons Manufacturing Co., Inc., Bonner Springs, KS, USA) and pelleted using a California Pellet Mill (CPM, Blaine, MN, USA). Crumble diets were fed during the starter phase, and pelleted diets were fed during both grower and finisher phases. All diets were formulated to be isonitrogenous and isocaloric by adjusting corn, soybean oil, and soybean meal. Basic nutrient specifications for whole dried BSFL used in this study are described in Table 1. Diet formulations and analyses are shown in Table 2. Three samples were collected from each diet: one from the beginning, middle, and end of the feed manufacturing batch. These three individual samples were mixed to form one composite sample to be analyzed. Moisture was analyzed using method FD016, Crude Protein was analyzed using method FD070, Fat was analyzed using method FD027, Acid Detergent Fiber was analyzed using method FD021, Metals and Minerals were analyzed using ME029 and ME069.

Table 1. Nutrient profile of dried whole black soldier fly larvae.

Nutrient	Concentration
Proximate Composition (% DM Unless Specified)	
Moisture (%)	3.46
Crude protein	42
Crude fat (acid hydrolysis)	36.2
Crude fiber	6.1
Acid detergent fiber	7.2
Neutral detergent fiber	15.1
Indispensable AAs (% DM)	
Arginine	1.73
Histidine	1.01
Isoleucine	1.54
Leucine	2.23
Lysine	2.18
Methionine	0.51
Phenylalanine	1.39
Threonine	1.25
Tryptophan	0.5
Valine	1.96

Table 1. *Cont.*

Nutrient	Concentration
Selected dispensable AA (% DM)	
Cystine	0.28
Selected fatty acids (% as-is)	
Lauric acid (C12:0)	12.3
Myristic acid (C14:0)	3.25
Palmitic acid (C16:0)	5.36
Oleic acid (C18:1)	5.56
Linoleic acid (C18:2)	6.18
Linolenic acid (C18:3)	0.53
Selected minerals	
Calcium (% DM)	1.49
Phosphorus (% DM)	0.81
Magnesium (% DM)	0.29
Sodium (% DM)	0.1
Potassium (% DM)	1.05
Iron (mg/kg DM)	135
Manganese (mg/kg, DM)	110
Zinc (mg/kg DM)	85.7
Copper (mg/kg DM)	8.5

Abbreviations: AA: amino acid; DM: dry matter.

Table 2. Nutrient composition of diets containing dried whole black soldier fly larvae.

	Starter			Grower			Finisher		
Ingredient, %	**Con**	**2.5% BSFL [3]**	**5.0% BSFL [3]**	**Con**	**2.5% BSFL [3]**	**5.0% BSFL [3]**	**Con**	**2.5% BSFL [3]**	**5.0% BSFL [3]**
Yellow Corn, Ground	51.50	51.99	52.65	57.65	57.93	58.61	61.25	61.41	62.06
Soybean Oil	1.54	0.75	0.02	1.74	0.98	0.21	3.07	2.26	1.49
Soybean Meal	43.53	41.40	39.05	37.78	35.80	33.45	33.11	31.30	28.96
BSFL	0.00	2.50	5.00	0.00	2.50	5.00	0.00	2.50	5.00
Calcium Carbonate	0.98	0.98	0.92	0.93	0.95	0.88	0.89	0.89	0.82
Dicalcium Phosphate	1.26	1.17	1.14	0.67	0.59	0.55	0.52	0.52	0.52
Sodium Chloride	0.38	0.37	0.37	0.39	0.39	0.39	0.39	0.38	0.37
L-Lysine	0.13	0.14	0.16	0.21	0.21	0.24	0.21	0.19	0.21
DL-Methionine	0.41	0.41	0.41	0.39	0.39	0.39	0.36	0.35	0.35
Threonine	0.12	0.14	0.15	0.11	0.12	0.14	0.06	0.06	0.08
Mineral Premix [1]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Vitamin Premix [2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Analyzed Nutrient Analysis									
Moisture, %	13.76	13.33	13.54	13.85	13.26	13.27	13.69	13.44	13.47
Dry Matter, %	86.24	86.67	86.46	86.15	86.74	86.73	86.31	86.56	86.53
Crude Protein, %	24.3	24	24	23.4	23.5	23.4	21.40	21.60	21.00
Crude fat, %	3.6	3.55	3.72	3.96	4.26	4.03	5.33	5.08	5.17
Ash, %	4.79	4.66	4.75	4.58	4.24	4.68	3.86	4.17	4.13
Fiber (Acid Detergent), %	3.5	3.8	5	3.3	3.5	2.3	2.30	2.60	2.70
Calcium, %	0.7	0.65	0.66	0.67	0.75	0.82	0.56	0.69	0.68
Phosphorus, %	0.63	0.61	0.56	0.54	0.56	0.54	0.51	0.50	0.50
Magnesium, %	0.17	0.17	0.16	0.17	0.17	0.17	0.16	0.16	0.16
Potassium, %	1.13	1.16	1.04	1.1	1.04	1.03	1.00	0.95	0.95
Sodium, %	0.11	0.12	0.11	0.12	0.19	0.16	0.12	0.14	0.14
Iron, ppm	154	142	136	123	126	144	108.00	96.80	107.00
Zinc, ppm	100	99.5	87.7	110	119	117	112.00	110.00	104.00
Manganese, ppm	97.2	98.5	85.9	116	131	134	119.00	127.00	104.00
Copper, ppm	20.2	20.5	18.1	17.8	22.2	23.6	21.30	19.00	16.90

Table 2. *Cont.*

Ingredient, %	Starter			Grower			Finisher		
	Con	2.5% BSFL [3]	5.0% BSFL [3]	Con	2.5% BSFL [3]	5.0% BSFL [3]	Con	2.5% BSFL [3]	5.0% BSFL [3]
Calculated Values									
TMEn, Kcal/Kg	3032	3033	3035	3124	3123	3122	3250	3249	3248
Lysine, %	1.54	1.54	1.54	1.45	1.45	1.45	1.31	1.31	1.31
Methionine, %	0.77	0.77	0.77	0.72	0.72	0.72	0.67	0.67	0.67
Threonine, %	1.14	1.14	1.14	1.04	1.04	1.04	0.91	0.91	0.91

[1] Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4 \bullet H_2O$), 60 mg; iron ($FeSO_4 \bullet 7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4 \bullet 5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium (Na_2SeO_3), 0.3 mg. [2] Vitamin mix provided the following (per kg of diet): Vitamin A, 8818 IU; Vitamin D3, 2480 IU; 25-hydroxyvitamin D3, 69 µg; Vitamin E, 35 IU; vitamin B12 (cobalamin), 15.5 µg; Biotin, 0.17 mg; Menadione, 1.98 mg; Thiamine, 1.87 mg; Riboflavin, 7.7 mg; d-Panthothenic Acid, 13.23 mg; Vitamin B6, 3.3 mg; Niacin, 44.1 mg; Folic Acid, 1.1 mg. [3] Black Soldier Fly Larvae sourced from EnviroFlight LLC.

2.5. Growth Performance

Group pen weights and feed consumption were measured weekly to calculate average body weight gain (ABWG), average feed intake (AFI), and Feed Conversion Ratio (FCR). All mortalities were also weighed and accounted for in growth performance metrics.

2.6. Necrotic Enteritis Challenge and Lesion Scoring

During Experiment 2, a subclinical necrotic enteritis challenge was induced. On days 19, 20, and 21, all pens were challenged with a broth culture of *C. perfringens* at approximately $1.0 \times 10^{8-9}$ concentration. Fresh inoculum was used each day. A field isolate originating from a southeastern commercial broiler operation was used as the challenge organism in this experiment. Inoculation volume was maintained for each pen. Inoculum was administered by mixing into the feed at the base of the feeder at a rate of 1 mL per bird, or 45 mL total per pen in Experiment 2. On day 21, three birds from each replicate pen were selected, sacrificed, weighed, and examined for presence of necrotic enteritis lesions. Scoring is based on a 0 to 3 score. Scoring details as follows: a score of 0 is given to normal intestines, a score of 1 is given for a slight mucus covering and loss of tone, a score of 2 is given for severe necrotizing enteritis, and a score of 3 is given for extreme necrotizing enteritis with blood in the lumen.

2.7. Statistical Analysis

Data were analyzed in JMP® 17 as a completely randomized design. Pen served as the experimental unit. Shapiro–Wilk normality test was conducted to ensure normality. Following normality testing, a one-way analysis of variance (ANOVA) was used to examine the fixed effect of BSFL dietary inclusion on growth performance. When statistical differences were observed, means were separated using Tukey's post hoc analysis and statistical significance was declared at $p < 0.05$. A Kruskal–Wallis test was utilized to analyze lesion scores.

3. Results

3.1. Growth Performance

Summary statistics for growth performance parameters are shown in Table 3. Average feed intake was consistent across all treatment groups in Experiment 1 and Experiment 2 ($p = 0.340$ and $p = 0.555$, respectively). Average Body Weight Gain (ABWG) was increased in birds provided 5.0% BSFL dietary inclusion compared to the control group in Experiment 1 ($p = 0.014$) and Experiment 2 ($p = 0.023$). No statistical differences were noted in ABWG of the 2.5% inclusion group when compared to the control, or when comparing 2.5%

and 5.0% inclusion rates. Feed Conversion Ratio (FCR) was statistically improved in the 2.5% inclusion rate group in Experiment 1 ($p < 0.001$) and Experiment 2 ($p < 0.001$) when compared to the control group. FCR was also improved in the 5.0% inclusion rate in Experiment 1 ($p < 0.001$) and Experiment 2 ($p < 0.001$) when compared to the control group. No statistical difference was noted when comparing FCR for 2.5% and 5.0% inclusion rates.

Table 3. Effect of Whole Dried BSFL [1,4] dietary inclusion on broiler growth performance.

Experiment 1	**Control**	**2.5% BSFL**	**5.0% BSFL**	**SEM** [2]	***p*** [3]
Day 0–42 AFI, kg	4.06	4.23	4.15	0.112	0.340
Day 0–42 ABWG, kg	2.48 b	2.57 ab	2.61 a	0.043	0.014
Day 0–42 FCR	1.59 a	1.55 b	1.53 b	0.012	<0.001
Experiment 2	**Control**	**2.5% BSFL**	**5.0% BSFL**	**SEM**	***p***
Day 0–42 AFI, kg	3.92	3.91	4.01	0.095	0.555
Day 0–42 ABWG, kg	2.73 b	2.88 ab	2.91 a	0.061	0.023
Day 0–42 FCR	1.44 a	1.37 b	1.38 b	0.053	<0.001

[1] Data were analyzed as a completely randomized design using JMP17 with cage serving as the experiment unit. Means were separated using Tukey's test for post-hoc analysis. $n = 10$. [2] SEM = highest standard error of the LS means pair-wise comparisons. [3] Means within rows with different lowercase letters differ $p \leq 0.05$ and are considered significantly different. [4] Black Soldier Fly Larvae sourced from EnviroFlight LLC.

3.2. Necrotic Enteritis Lesion Scores

Summary statistics for Necrotic Enteritis Lesion Scores are shown in Table 4. Overall, lesion scores remained below an average of 1 for all treatment groups. The highest lesion scores were found in the control group (0.64), while 2.5% and 5.0% treatment groups had lower average lesion scores (0.43 and 0.5, respectively). Upon analysis, no statistical differences were found among treatment groups.

Table 4. Effect of BSFL [3] dietary inclusion on subclinical NE [4] lesion scores and mortality.

Experiment 1	**Control**	**2.5% BSFL**	**5.0% BSFL**	**SEM** [2]	***p*** [1]
Day 0–42 Overall Mortality, %	6.50	10.00	7.50	2.386	0.449
Experiment 2	**Control**	**2.5% BSFL**	**5.0% BSFL**	**SEM**	***p***
Day 21 Lesion Scores (0–3)	0.64	0.43	0.50	0.168	0.730
NE Specific Mortality, %	7.11	4.00	4.22	1.683	0.230
Day 0–42 Overall Mortality, %	11.69	13.33	11.87	2.134	0.791

[1] Data were analyzed as a completely randomized design using JMP17 with cage serving as the experiment unit. Means were separated using Tukey's test for post-hoc analysis. $n = 10$. [2] SEM = highest standard error of the LS means pair-wise comparisons. [3] Black Soldier Fly Larvae sourced from EnviroFlight LLC. [4] Necrotic enteritis.

3.3. Mortality

Summary statistics for necrotic enteritis mortality and overall mortality are shown in Table 4. Overall, no statistical differences were noted among treatment groups in either experiment. Numerically, necrotic enteritis mortality was highest in the control group (7.11%) compared to the 2.5% inclusion rate (4.00%) and the 5.0% inclusion rate (4.22%).

4. Discussion

Growth performance results showed that BSFL ingredients were able to improve feed efficiency in broilers. This trend was consistent among the two different experiments. There were slight differences in FCR between Experiment 1 and Experiment 2, but it is important to remember that these were different cohorts of animals, reared in different environments, and with different pen sizes. These natural differences, along with potential differences

in parent stock, explain subtle differences in growth performance when comparing Experiments 1 and 2. ABWG was increased with BSFL inclusion while feed intake remained constant. This led to an improved efficiency at 2.5% and 5.0% inclusion rates compared to the control groups. In another study by Vilela et al. [5], whole dried BSFL also showed improvements in feed efficiency when added to the diets, but at much higher inclusion rates, including 10% and 20%. Although 2.5% and 5.0% rates were tested in that study, these lower inclusion rates did not impact overall performance. Although the ingredients were similar in both experiments, differences in growth performance improvements may be due to different suppliers, or different BSFL nutrient composition between the two experiments. These differences in BSFL nutrient composition were highlighted in a recent study which fed BSFL to broilers at similar feeding rates. Results from this study indicated that nutrient composition of BSFL varied widely by source, and that ingredient source played a significant role in the presence or absence of growth performance improvements in broilers [17]. This notable variability in ingredient composition highlights the need for researchers to fully understand and analyze their BSFL ingredients prior to formulating experimental diets.

After the intestines were examined at d21, lesion scoring results showed a numerical trend towards lower lesion scores in birds fed 2.5% and 5.0% BSFL compared to those fed the control diet. Prior to this study, most studies of feeding BSFL to poultry have focused on insect meal or insect oil. Additionally, disease challenge models in broilers fed BSFL have been somewhat limited. The use of whole dried BSFL in combination with a subclinical necrotic enteritis model makes this study vital to future works. In a previous study by Singha et al. [18], whole dried BSFL and defatted BSFL meal were both shown to decrease intestinal inflammation after induction of intestinal enteritis in rainbow trout. These findings, in combination with the supporting literature, indicate that BSFL may improve intestinal health and integrity during the presence of a subclinical enteric disease challenge; however, further testing is needed to solidify these findings. In future studies, increased sample size or a more severe challenge model may be utilized to emphasize these potential differences.

Although overall mortality was not impacted by BSFL inclusion rate, a numerical increase occurred in the 2.5% inclusion group compared to the control. Although the cause of this numerical increase is unknown, it is important to note that each pen contained only 20 birds, so even small numbers of mortality can have large impacts on mortality data. Additionally, a numerical reduction in percent of necrotic enteritis mortality was noted when BSFL was included in the diet at both 2.5% and 5.0% when compared to the control. When challenged with *Salmonella gallinarum*, low levels of BSFL supplementation aided in livability, and demonstrated an increase in CD4+ lymphocyte proliferation, suggesting a T helper cell dominated response [19]. In 2025, researchers demonstrated that the use of BSFL in broilers at 5% inclusion also helped growth performance during a necrotic enteritis challenge model; however, this paper utilized defatted BSFL meal instead of whole dried BSFL, which is used in the current study [20]. These results align with those from other species fed BSFL ingredients such as rainbow trout, which demonstrated decreased mortality after response to a disease challenge [6].

Further investigation is warranted to determine the mode of action responsible for these important statistical improvements in growth performance, coupled with interesting numerical differences in disease outcomes. Regarding numerical differences in overall mortality and necrotic enteritis specific mortality, more replication may be needed to better detect subtle differences in health outcomes, which could support more substantive conclusions. Perhaps a stronger, clinical enteritis challenge, might be used to determine if any health improvements potentially exist. Additionally, it is important to remember

that these studies were conducted using two different cohorts of animals. In an ideal setting with ideal facilities, these studies could be conducted simultaneously in a factorial design, which might also help to understand some of the subtle differences seen in the current study.

5. Conclusions

Overall, the results of this study, paired with the regulatory approval for the use of this ingredient in poultry diets, indicates that whole dried BSFL are safe and effective for inclusion in broiler diets at both 2.5% and 5.0% inclusion rates. When exposed to a subclinical necrotic enteritis challenge, whole dried BSFL numerically improved lesion scores and NE-specific mortality as determined by a licensed veterinarian. These results clearly justify the use of BSFL as a feed ingredient in broiler diets and highlight the need for and importance of further investigations into potential functional properties associated with this ingredient. Further investigation is warranted to understand optimal inclusion rates with and without disease challenges.

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

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Conflicts of Interest: Author Daniel Adams and Elizabeth Koutsos were employed by the company EnviroFlight, LLC. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

FCR	Feed Conversion Ratio
AFI	Average Feed Intake
ABWG	Average Body Weight Gain
BSFL	Black Soldier Fly Larvae
NE	Necrotic Enteritis
ANOVA	Analysis of Variance
AA	Amino Acid
DM	Dry Matter

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