Journal of Veterinary Science and Animal Husbandry
Volume 13 | Issue 1
ISSN: 2348-9790

Research Article Open Access

Effect of Black Soldier Fly Larvae Meal Based Diet on Performance of Broiler Chickens during Finishing Phase

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Citation: Domitila Njoki, James K.Tuitoek, Anthony Macharia King'ori, Mary Kivali Ambula, Haron Juma Masai (2025) Effect of Black Soldier Fly Larvae Meal Based Diet on Performance of Broiler Chickens during Finishing Phase, J Vet Sci Ani Husb 13(1): 102

Received Date: February 04, 2025 **Accepted Date:** March 04, 2025 **Published Date:** March 10, 2025

Abstract

The present study has evaluated the effects of different inclusion levels of full fat black soldier fly (BSF) *(Hermetia illucens L.)* larva meal on the growth performance, carcass yield, organ development and gut pH of broiler chickens. A total of ninety Cobb 500 broiler chicken of mixed sex were reared from day 21 to day 49 and assigned to 5 dietary treatments replicated thrice with 6 birds per replicate. The BSF larva meal was included at varied levels (0, 7.5%, 15%, 22.5% and 30%) in isonitrogenous and isoenergetic diets formulated for finisher feeding phase. One chicken per pen were slaughtered at day 49 for evaluation of carcass yield, organ development and gut pH. Data was analyzed using Statistical Analysis System software (SAS, 2009) and significant means were separated using Tukey Honest Significant Difference (HSD). The results showed that the total and weekly feed intake was not affected. Live, dressed, carcass weights, total weight gained, average daily weight gain showed a significant ($P<0.05$) quadratic response to BSF larvae meal to a maximum of 22.5% while feed conversion ratio (FCR) and protein efficiency ratio (PER) was optimal at 15% inclusion level. The thigh and drumstick portion posted a significant ($P<0.05$) positive linear and quadratic respectively. In addition, the trial diets significantly affected the weight of the liver, spleen and gizzard. However, the inclusion of the larvae meal did not have significant effect ($P>0.05$) on feed intake, relative cold weight, organ weight for the heart and bursa as well as the pH for the crop, proventriculus, duodenum, jejunum and ileum. Therefore, BSF larvae meal can be included up to 22.5 % in broiler finisher diets because a higher level of inclusion results in poorer growth performance. The optimal protein efficiency ratio and feed conversion ratio was attained at 15% inclusion level. The authors recommend that the composition of rearing substrates for BSF larvae should be manipulated to produce larvae with high CP contents (≥45%) and relatively low-fat contents and the level of inclusion of BSF larvae meal to replace soybean meal in broiler finisher diets should not exceed 22.5% for optimal growth performance.

Keywords: Black soldier fly larvae meal, broiler, feed intake, live weight, ADG, FCR, PER, internal organs, gastrointestinal tract pH

Introduction

Broiler production is one of the economic ways for protein supply for human population. Feed account for at least 70% of production cost in broiler production and the protein fraction bears a bigger proportion of that cost compared to the other nutrients. This makes the enterprise less profitable. Therefore, any reduction in the price of the protein source will have a positive impact on the cost of broiler production. Assessment of non-conventional feed ingredients in chicken production has been carried out resulting in a shift from plant and animal protein sources to insect-based protein sources [24]. One potential source of insect protein is the larvae of black soldier fly. The meal from black soldier fly larvae (BSFL) has been suggested as a sustainable alternative protein source to soybean and fish meal [12]. The nutrient composition of BSFL meal makes it an attractive ingredient in monogastric animal feed, particularly due to its favorable essential amino acid profile and high crude protein content (up to 57.6%) [16]. Compared with soybean and fish meal, BSFL meal tends to contain high fat and crude fibre content. It also contains high levels of oleic fatty acid but deficient in arginine amino acid. On the other hand, soy bean meal limited in sulfur amino acids (methionine and cystine) and contains trypsin inhibitor that hinders the activity of the proteolytic enzyme's trypsin and chymotrypsin in monogastric animals.

By the year 2023, International Centre of Insect Physiology and Ecology (ICIPE) documented that 1200 farmers were rearing BSF but data on the amount of meal being produced was not available. Another baseline survey report by the Ministry of Agriculture and Livestock development in Kenya indicated that the farmers ranged between small scale and large scale while the Association of Kenya Feed Manufacturers indicated that there was no adequate amount of the BSF larvae for commercial feed millers hence upscaling in production was necessary.

The safety of the larvae meal by being free from *Salmonella spp* and *Escherichia coli* irrespective of the rearing substrate used has been demonstrated and documented [20]. In addition to the nutritional and functional benefits of BSF larvae meal, its use in broiler feed could help reduce the environmental footprint of poultry feed production and close the gap in a circular food economy. Black soldier fly larvae consume food waste and convert it into high-quality protein and fat [4]. However, the crude protein (CP) of BSF larvae meal varies and has been found to be dependent on the proximate composition of the rearing substrate. Its crude protein content has been reported to range from 13.59% when reared on a substrate comprising of 50% banana peels and 50% cassava peels to 57% when reared on turkey chick feed [26]. However, CP content in the larvae can be maximized by manipulating the nutritive composition of the rearing substrate. On the other hand, the fat contents vary greatly with as low as 8.39 % when reared on liver and as high as 57% when reared on bread [16]. This restricts inclusion levels in chicken diet because high fat content reduces feed intake in chicken [20]. When the larvae CP content is high, then the level of fat reduces since their contents have an inverse relationship [16]. Marono *et al.* [22] documented that BSF larvae meal post a positive correlation between CP level and CP digestibility indicating that, as the amount of crude protein increases, the crude protein digestibility increases. This could be explained by the negative correlation between crude protein content and acid detergent fibre (ADF) or acid detergent insoluble protein (ADIP). The interpretation of the results from that particular study is that that when CP level of BSF larvae increases, the amounts of ADF and nitrogen linked to ADF decrease and ultimately affect the digestibility of crude protein positively.

The development of vital organs namely heart, liver, spleen and bursa are necessary for the many body functions and processes needing maintenance in the production of healthy birds. The spleen and bursa of fabricius are both lymphoid organs which are part of the avian immune system. The bursa is the only lymphoid organ that acts as both a primary and secondary lymphoid organ in avian species. It is therefore of importance to evaluate the development of the bursa mass and the spleen mass, as well as the ratio between them, to determine the level of infectious activity the bird has been exposed to throughout its lifetime [9]. Moreover, feed consumed by broilers has a bearing on development and pH value of different segments of the gastrointestinal tract (GIT). The pH value is optimal for each segment and any disruption from the norm will compromise growth of microbes, efficiency of digestive enzymes, digestion, and absorption of nutrients and overall performance of the chicken [21]. Therefore, any ingredient introduced to chicken as feed should be monitored so as to maintain normal pH and therefore avoid adverse effect on the overall performance of the chicken being fed.

The inclusion level of BSF larvae meal in the broiler diets is still debatable with varying levels documented by different researchers. The limitation in inclusion has been pegged on high fat and crude fibre content in the larvae meal. Since the CP content can be maximized through manipulation of the rearing substrate the BSF larvae used in this study were reared using a specially formulated rearing substrate so as to maximize the CP (48%) content and consequently minimize the fat (23%) content, increase digestibility with expectation of 100% replacement of soybean meal in the broiler feed.

The objective of this study was to establish the optimal inclusion level of high- density CP BSFL meal in broiler chicken. The effects of replacing soybean meal with BSF larvae meal on feed intake, growth performance, carcass yield, organ development and gastro intestinal tract pH was evaluated.

Limitation of the Study

The study focused on proximate composition (crude protein, fat, ash, crude fibre) of the meals of soy bean and BSF larvae without assessing other important micronutrients such as vitamins, minerals, and amino acid profiles and fatty which may vary more so in the BSF larvae meal due to variation in its nutritive composition partially attributed to the rearing substrate. A broader nutrient analysis should be conducted to determine the effect of the CP content in the BSF larvae meal on amino acid and fatty acids. The study relied on BSF larvae meal which was obtained through modulation of the proximate composition of the rearing substrate with the aim of optimizing the CP content of the larvae and growth performance traits.

Materials and Methods

Study Site

The experiment was conducted at the University of Nairobi, Animal Production Department Poultry Unit. It is located at latitude 1.25287867437 and Longitude of 36.7298431783. The area receives 1200 mm of rainfall per annum with bimodal pattern from March to July and October to December. The temperature ranges between 12^0C and 28^0C.

Source of BSFL meal

The BSF larvae used in this study were reared in three batches in a climatical chamber in a private farm (Zihanga) specializing in commercial production of BSF. The farm is located in Lower Kabete in Kiambu County, Kenya. The BSF larvae were reared on specifically formulated substrate as determined in an earlier experiment with the aim of optimizing the CP content. The nutritive composition of the rearing substrate and the corresponding BSF larvae is illustrated in Table 1.

Table 1: Analyzed proximate composition (%) of the rearing substrate and the corresponding BSF larvae meal

Nutrient	Rearing substrate	BSF larvae meal
Dry matter	90±1	94.97
Crude protein	15±1	48.5
Crude fibre	17±2	16.2
Crude fat	7±2	23.5
Crude ash	7±2	12.31

Non fibrous carbohydrate	50±5	_
ME Kcal/kg	2800 ± 100	3200 ± 100

Experimental Diets

There were five iso-caloric and iso-nitrogenous diets formulated using varying levels of BSFL meal to replace soybean meal to meet the National Research Council requirements for broiler chickens during the finishing phase; 3100 Kcal/kg and 20% CP (N-RC, 1994). Eighty percent of de-oiled soybean meal was mixed with 20% of full fat soybean meal to attain the CP content equivalent to that of BSF larvae meal. The BSF larvae meal inclusion levels were 0, 7.5, 15, 22.5 and 30% and replaced equivalent amounts of soybean meal mixture. The experimental diets are presented in Table 2.

Table 2: Proximate composition of the experimental diets for the broiler chicken

Ingredient (%)	T1	T2	T3	T4	T5
Maize grain	65.0	65.0	65.0	65.0	65.0
BSFL meal[1]	0	7.5	15.0	22.5	30.0
Soya bean meal[2]	30.0	22.5	15.0	7.5	0
Lysine	0.4	0.4	0.4	0.4	0.4
Methionine	0.3	0.3	0.3	0.3	0.3
DCP (granular 24%)	0.5	0.5	0.5	0.5	0.5
Limestone	2.0	2.0	2.0	2.0	2.0
Salt	0.2	0.2	0.2	0.2	0.2
Premix[3]	0.3	0.3	0.3	0.3	0.3
Calculated nutrients					
Crude Protein	19.6	19.6	19.7	19.7	19.8
ME (Kcal/kg)	3176	3159	3152	3146	3138
Ether Extract	4.9	6.1	7.1	8.2	9.2
Crude Fibre	6.0	6.7	7.4	8.1	8.9
Ash	2.4	2.7	2.8	3.2	3.4

BSFL; Black soldier fly larvae, Soy bean meal; 20% full fat, 80% de-oiled, Premix; Supplied the following per 2Kg of diet: Each 2Kg contains: Vitamin A, 8,500,000 IU; Vitamin D3, 1,600,000 IU; Vitamin E, 4,000 IU; Vitamin K3, 2,000 mg; Vitamin B2, 5,000 mg; Vitamin B3, 20,000 mg; Vitamin B5, 8,800 mg; Vitamin B6, 1,200 mg; Vitamin B9, 00 mg; Vitamin B12, 8 mg; Chlorine chloride, 200,000 mg; Antioxidant, 125,000 mg; Fe, 5,000 mg; Mn, 80,000 mg; Zn, 50,000 mg; Cu, 2,000 mg; I, 1,200 mg; Co, 200 mg; Se, 100 mg.

Chemical Analysis

The BSF larvae meal, maize grain and soybean meal used in the current study were analysed for DM, CP, fat, fibre and ash before being incorporated into the experimental diets. Dry matter (DM) was determined by drying in a hot air oven at 105°C for 24 h following standard method 925.09 (AOAC, 2006), Ash by burning the samples in a muffle furnace at 550°C for eight hours following standard method 923.03 (AOAC, 2006), ether extract by the Soxhlet method (using ether) following standard methods 920.39 (AOAC, 2006). Total nitrogen for crude protein (N x 6.25) determination was obtained using the micro-Kjeldahl method following standard methods 920.87 (AOAC, 2006).

Broiler Chicken and Management

One hundred and fifty, day old broiler chicks of mixed sex (Cobb-500) obtained from Ken-Chick® Ltd were used in this experiment. The chicks were reared under conventional brooding conditions, receiving 24 hours of light and a temperature of 30-34° C during the first 21 days of age. They were fed on conventional commercial broiler starter crumbs sourced from one of the reputable feed manufacturers in Kenya during this period. The brooding temperature was maintained using infra-red electric bulbs and gradually adjusted to room temperature towards the end of the third week. On day 1 and day 18, all birds were vaccinated against Newcastle Disease (NCD) and Infectious Bronchitis (IB) respectively and against Infectious Bursal Disease (IBD) on day 7 and day 14 [2].

On the 21st day of age, the chicks were weighed individually and 90 of them, weighing between 885 and 890 grams were randomly assigned to 15 experimental cages (1 by 1 m^2) in a deep litter system. Each cage was equipped with a round feeder and a semi-automatic water drinker. Each cage with six birds was the experimental unit. The five broiler diets were then randomly assigned to the experimental units in a CRD arrangement with three replications per treatment and fed on the experimental diets for 28 days experimental period. Throughout the experimental period, birds were offered *ad libitum* access to feed and clean drinking water.

Data collection

Feed Intake, Growth Rate, Feed Conversion Efficiency and Protein Efficiency Ratio

Data was collected for 28 days. On a daily basis, broilers were offered known amounts of feed and the leftovers were collected before the next feeding. Additionally, birds were weighed at 7 days intervals. The average body weight gain was calculated by dividing the mean total body weight gain per bird by 28 days. Weekly feed intake was determined as the difference between feed offered and feed refusal for one week. Feed intake per bird was the total feed consumed per week divided by the number of birds per pen. Total feed intake per bird was obtained as the summation of weekly average feed intake per bird for each diet during the experimental period. Feed conversion ratio (FCR) was calculated as the ratio of cumulative feed intake (g) per bird to the average live weight gain per bird during the experimental period. The protein quality was evaluated on basis of Protein Efficiency Ratio PER) which is an expression of weight gained per protein consumed in grams for the experimental period. Weekly and overall growth rates, feed intake, feed conversion ratio and PER were calculated.

Carcass Data

At the end of the experimental period, one bird from each of the fifteen pens was selected, isolated and starved overnight with free access to water. The birds were killed by cutting the throat behind the lower jaw with a razor-sharp knife. Each chicken was eviscerated (this included the removal of all the internal organs, feet and neck) and the weight of the warm carcass was recorded. Dressing percentage was calculated as the percentage difference between the live weight of the chicken and the weight of the cold carcass. The weights of the commercial portion yields namely; thigh, drumstick and breast were taken. The thigh and drumstick were removed by cutting above the thigh towards the acetabulum and behind the pubic bone. The drumstick and thigh were separated by cutting perpendicular towards the joint connecting these two cuts. The carcasses were then stored for 24 hours at 4°C and the cold carcass weight recorded. Percentage portion yields were then calculated by expressing these weights as a percentage relative to warm dressed carcass weight.

Internal Organs Data

Immediately following slaughter, the weights of the heart, spleen, liver, gizzard and bursa of fabricius were taken. The organ weight relative to the live weight of the chicken at slaughter was expressed as a percentage. The weight ratio of spleen to bursa of fabricius was also calculated.

Gastrointestinal Tract Ph Data

The pH of the gastrointestinal segments; the crop, duodenum (on the gizzard side of the duodenum at the start of the pancreas), jejunum (approximately in the centre) and the ileum (5mm from Meckel's diverticulum to the ileocecal junction) within 15 minutes following slaughter. The pH was measured using a calibrated (standard buffers pH 4.0 and 7.0 at 25°C) portable Crison pH 25 meter (Alella, Barcelona) by inserting the pH electrode into the centre of the GIT segment being measured. The probe was thoroughly rinsed with distilled water between each reading. The probe was rested in a KCl 3M electrolytic solution when not being used or rinsed.

Statistical Analysis

The data collected for parameters under investigation were subjected to one-way analysis of variance (ANOVA) in a completely randomized design (CRD) using SAS procedures, (2009). Means were separated using Tukey's HSD at 5% significance level. All the significant responsive parameters were further subjected to polynomial regression analysis to establish the type of dose response/relationship between the BSF larvae inclusion level and the parameter under investigation.

The statistical analysis was based on the following illustrated equation.

Statistical Model

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$

Where:

Y_{ij} = Observation (feed intake (FI), live weight, weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), carcass yield, organ weight, pH of GIT segment)

μ = Overall mean

T_i = Inclusion level of BSFL meal (0, 7.5, 15, 22.5 and 30%)

ε_{ij} =Random error component.

Results

Feed Intake, Growth Rate, Feed Conversion Efficiency and Protein Efficiency Ratio

The results of the above performance indicators are presented in Table 3. The measurements for these parameters were feed intake (FI), live weight (LW), daily gain (ADG), feed conversion ratio (FCR) and protein efficiency ratio (PER). The dietary inclusion level of BSF larvae meal had a significant ($P<0.05$) effect on final live weight, average daily gain, FCR and PER. The live weight was also significantly ($P< 0.05$) affected during the first and fourth week of the study when the 15% and 22.5% inclusion level had the highest live weights while the control diet (0% BSF larvae meal inclusion) had the lowest during the second and third week. However, feed intake was not affected (P>0.05) by the inclusion level of BSF larvae meal in the broiler finisher diets.

Table 3: The means (± standard error) of weekly live weight (g), total weight gain, average daily weight gain (g) weekly feed intake (g), total feed intake (g), feed conversion ratio (FCR) and protein conversion efficiency (PER) as influenced by inclusion of black soldier fly larvae meal (BSM) in broiler chicken diets during week 4, 5,6 and 7 of age

	Dietary level of BSFL meal						
Parameter	T1 - 0%	T2 - 7.5%	T3 - 15%	T4 - 22.5%	T5 - 30%	SEM	P value
Weight week 4	1342±3.25^{a}	1436±6.06^{b}	1476±2.17^{c}	1461±5.51^{c}	1430±4.41^{b}	±12.571	0.001
Weight week 5	1983±38.46^{a}	2086±18.51^{a}	2141±35.42^{a}	2160±71.76^{a}	2079.17±32.76^{a}	±23.13	0.104NS
Weight week 6	2639±58.95^{a}	2808±7.17^{a}	2906±45.24^{a}	2869±113.86^{a}	2710±37.23^{a}	±35.69	0.67NS
Weight week 7	3064±90.62^{a}	3331±25.93^{bc}	3499±.33^{c}	3382.33±136.67^{bc}	3229.69±54.91^{ab}	±50.53	0.020
Weight Gained	2180±90.28^{a}	2441.67±23.55^{ab}	2579.56±30.79^{b}	2587.58±136.62^{b}	2343±53.93^{ab}	±44.91	0.020
ADG g	77.67±2.9^{a}	87.33±0.88^{ab}	92.12±0.26^{b}	92.41±2.17^{b}	83.67±2.03^{ab}	±9.344	0.020
FI week 4	876±6.66^{a}	895.33±20.2^{a}	936±4.1^{a}	925.0±36.04^{a}	907±7.8^{a}	±9.21	0.26NS
FI week 5	1113.75±33.01^{a}	1106.58±29.28^{a}	1118.3±5.51^{a}	1160.0±59.64^{a}	1073.33±49.53^{a}	±16.83	0.60NS
FI week 6	1306.25±23.85^{a}	1310.5±15.98^{a}	1317.92±23.72^{a}	1263.67±52.41^{a}	1204.0±40.0^{a}	±0.17a	0.173NS
FI week 7	1438.67±29.87^{a}	1390.0±17.04^{a}	1547.33±26.34^{a}	1529.29±45.78^{a}	1473.67±48.22^{a}	±20.47	0.056NS
Total FI	4735±73.45^{a}	4703±49.8^{a}	4920±11.36^{a}	4878±120.75^{a}	4659±136.65^{a}	±43.85	0.25NS
ADFI, g	169.10±2.62^{a}	168.0±1.76^{a}	175.71±0.40^{a}	174.21±4.31^{a}	166.36±4.88^{a}	±1.56	0.25NS
FCR week 5	1.79±0.028^{a}	1.81±0.007^{a}	1.84±0.006^{a}	1.81±0.066^{a}	1.85±0.036^{a}	±0.015	0.757NS
FCR week 7	2.18±0.04^{b}	1.93±0.01^{a}	1.88±0.01^{a}	1.96±0.07^{a}	1.99±0.04^{a}	±0.03	0.001
CP Intake	946.93±14.69^{a}	940.60±9.96^{a}	984.00±^{a}	975.6±24.15^{a}	931.00±27.33^{a}	±8.77	0.254NS
PER	2.3±0.06^{a}	2.60±0.02^{b}	2.66±0.03^{b}	2.56±0.11^{b}	2.52±0.05^{ab}	±0.04	0.001

^{a,b,c} Means with different superscripts within the same row differ significantly (P<0.05)

Carcass Yield

The carcass yield results are presented in Table 4. Inclusion of BSF larvae meal significantly (P<0.05) affected live slaughter weight, dressed weight and cold carcass weight. The dressing percent carcass yield was not affected significantly (P> 0.05) by inclusion levels of BSF larvae meal. The inclusion of BSF larvae meal significantly (P <0.05) affected the weight of the thigh and drumstick but had no effect on the weight of the breast portion yield (P>0.05). Regarding the relative weight of the portion yield to dressed carcass weight, the inclusion of the larvae meal had negative significant (P<0.05) effect on the breast portion but did not have a significant (P>0.05) influence on the thigh and drumstick. The effect on the fat pad was significant (P<0.05) with linear response with increase in BSF larvae meal in the trial diets.

Table 4: The means (± standard error) of live weight, warm carcass weight, cold carcass weight, dressing percentage and carcass portion yield of broilers as influenced by inclusion of black soldier fly larvae (BSFL) meal during the finishing phase

	Treatments (BSFL meal inclusion level)						
Parameter	T1 - 0%	T2 - 7.5%	T3 - 15%	T4 - 22.5%	T5 - 30%	SEM	P value
Live weight	3098±59.55^{a}	3278±28.04^{ab}	3456±34.80^{b}	3420±59.51^{b}	3333±95.37^{ab}	±40.75	0.013
Warm carcass wgt	2145.3±49.94^{a}	2342.0±80.21^{ab}	2480.0±87.16^{ab}	2624.0±68.09^{b}	2500.0±106.01^{ab}	±53.17	0.016
Cold carcass wgt	2089.0±53.68^{a}	2243.0±105.29^{ab}	2377.33±81.83^{ab}	2522.0±46.20^{b}	2371.33±113.33^{ab}	±50.33	0.042
Fat pad grams	16±4.34^{a}	38.77±3.16^{b}	35.53±3.99^{b}	39.47±2.63^{b}	62.50±4.82^{c}	±4.13	0.001
Dressing %	68±1.29^{a}	67±2.6^{a}	69±2.4^{a}	73±1.2^{a}	71±1.5^{a}	±0.9	0.28NS
Drumstick weight	130.77±10.46^{a}	155.5±5.46^{ab}	158.4±9.59^{ab}	167.97±2.73^{b}	153.6±4.47^{ab}	±4.26	0.044
Thigh weight g	162.67±4.2^{a}	180.40±2.31^{ab}	176.73±9.9^{ab}	193.87±4.05^{b}	203.93±7.39^{b}	±4.47	0.007
Breast weight g	930.47±14.45^{a}	933.33±47.50^{a}	989.8±41.28^{a}	1047.33±35.63^{a}	929.4±59.94^{a}	±20.32	0.425NS
R^{1} %Drumstick	6.08±0.31^{a}	6.64±0.06^{a}	6.37±0.17^{a}	6.41±0.09^{a}	6.15±0.19^{a}	±0.09	0.373NS
R^{1} %Thigh	7.58±0.52^{a}	7.72±0.29^{a}	7.14±0.39^{a}	7.39±0.16^{a}	8.16±0.16^{a}	±0.13	0.108NS
R^{1} % Breast	43.40±0.76^{b}	39.81±0.65^{ab}	39.91±0.92^{ab}	39.90±0.40^{ab}	37.11±0.91^{a}	±0.61	0.003

^{a,b} Means with different superscripts within the same row differ significantly (P<0.05)

Internal Organs

The organs under evaluation were liver, heart, gizzard, spleen and bursa of fabricius and the results are presented in Table 5. The inclusion of BSF larvae meal in the diets of broiler finisher diets had significant (P <0.0 5) impact on the weight of the gizzard, liver and spleen. The weight of the heart and bursa of fabricious were not significantly (P >0.05) affected as well as the ratio of spleen to bursa of fabricious. The organs relative weight to the live weight were not affected (P>0.05) by the inclusion of the larvae meal in the broiler diet.

Table 5: Mean (± standard error) of organ weight and organ weight relative to live body weight as influenced by inclusion of black soldier fly larvae (BSFL) meal in broiler chicken diets during the finishing phase

	Treatments (BSFL meal inclusion level)						
Organ	T1 - 0%	T2 - 7.5%	T3 - 15%	T4 - 22.5%	T5 - 30%	**SEM**	***p value***
Liver	45.53±1.13^{a}	57.87±1.79^{ab}	62.23±5.66^{b}	61.93±3.59^{b}	60.30±0.75^{b}	±2.05	0.02
Spleen	2.97±0.3^{a}	4.4±0.63^{b}	4.47±0.41^{b}	4.53±0.30^{b}	3.12±0.23^{a}	±0.39	0.04
Bursa	1.37±0.18^{a}	2.63±0.38^{a}	3.5±1.05^{a}	2.1±1.17^{a}	2.1±0.21^{a}	±0.34	0.39NS

Spleen: Bursa ratio	2.19±0.11^{a}	1.8±0.44^{a}	1.44±0.29^{a}	2.16±0.48^{a}	1.52±0.07^{a}	±0.76	0.15NS
Heart	15.27±1.03^{a}	15.5±0.67^{a}	17.53±2.19^{a}	19.93±1.46^{a}	18.1±2.0^{a}	±0.76	0.27NS
Gizzard	47.0±3.26^{a}	54.57±2.18^{ab}	53.1±1.27^{ab}	69.4±6.58^{b}	51.5±3.58^{a}	±2.49	0.019
R^{1} %liver LW^{2}	1.47±0.063^{a}	1.77±0.063^{a}	1.80±0.177^{a}	1.81±0.11^{a}	1.81±o.05^{a}	±0.052	1.66NS
R^{1} % spleen LW	0.10±0.01^{a}	0.13±0.02^{a}	0.13±0.01^{a}	0.10±0.01^{a}	0.12±0.06^{a}	±0.007	0.137NS
R^{1} % bursa LW	0.04±0.007^{a}	0.08±0.01^{a}	0.1±0.03^{a}	0.06±0.03^{a}	0.07±0.01^{a}	±0.01	0.439NS
R^{1} % heart LW	0.49±0.04^{a}	0.47±0.02^{a}	0.51±0.06^{a}	0.58±0.05^{a}	0.54±0.06^{a}	±0.02	0.555NS
R^{1} % gizzard LW	1.51±0.04^{a}	1.61±0.079^{a}	1.54±0.78^{a}	2.03±0.21^{a}	1.55±0.16^{a}	±0.07	0.064NS
Spleen^{2} : Bursa LW	0.07±0.002^{a}	0.06±0.01^{a}	0.04±0.01^{a}	0.18±0.03^{a}	0.05±0.002^{a}	±0.03	0.412 NS

^{a,b} Means with different superscripts within the same row differ significantly ($P<0.05$)

Gastrointestinal Tract Data (pH)

The pH in the gizzard was significantly ($P<0.05$) affected by inclusion of BSF meal in the diets but there was no significant ($P>0.05$) effect on the pH of the crop, proventriculus duodenum and ileum as presented in Table 6.

Table 6: Mean (± standard error) of gastrointestinal tract segments pH values as influenced by inclusion of black soldier fly larvae (BSFL) meal in broiler chicken diets at finishing phase

		Inclusion level of BSFL Meal						
Organ	Normal pH	T1 - 0%	T2 - 7.5%	T3 - 15%	T4 - 22.5%	T5 - 30%	SEM	*p* value
Crop	6.084^{1}	4.70±0.82^{a}	5.13±9.37^{a}	4.74±1.05^{a}	5.23±0.23^{a}	3.73±1.13^{a}	±0.71	0.71NS
Proventriculus	4.65^{1}	3.2±0.06^{a}	4.3±0.52^{a}	3.9±0.37^{a}	4.64±0.16^{a}	3.53±0.63^{a}	±0.29	0.174NS
Gizzard	3.47^{1}	2.17±0.03^{a}	3.31±0.10^{b}	2.88±1.30^{ab}	3.08±0.41^{ab}	2.08±0.29^{a}	±0.16	0.014
Duodenum	5.5-6.2^{2}	4.84±0.40^{a}	4.66±0.33^{a}	4.97±0.37^{a}	4.58±0.14^{a}	5.05±0.52^{a}	±0.88	0.88NS
Jejunum	5.8-6.9^{2}	5.3±0.16^{a}	5.04±0.17^{a}	5.52±0.16^{a}	4.93±0.30^{a}	5.51±0.35^{a}	±0.12	0.36NS
Ileum	6.3-8.0^{2}	6.17±0.31^{a}	6.0±0.49^{a}	5.98±0.22^{a}	5.67±0.15^{a}	6.2±0.20^{a}	±0.13	0.73NS

^{a,b} Means with different superscripts within the same row differ significantly ($P<0.05$)

Polynomial Regression Analysis

Polynomial regression analysis was carried out for parameters which were statistically significant ($P<0.05$). The linear, quadratic and cubic relationships between the parameter under investigation and the level of inclusion of BSF larvae meal in the diets were established. The results of these relationships are presented in Table 7. In addition, the dose relationships between the level of BSFL meal, FCR and PER was plotted as indicated in Figures 3 and 4.

Table 7: Polynomial response for significantly different parameters under investigation

Parameter	Pooledp value	LinearP Value	QuadraticP value	CubicP value	R^2
Final live weight (21-49d) g	0.013	NS	0.002	NS	64.7
Weight Gained (28 days)	0.020	NS	0.002	NS	64.8
ADG (28 days)	0.020	NS	0.002	NS	64.8
FCR (21-49 days)	0.001	NS	0.001	0.001	82.4
PER (21-49 days	0.001	NS	0.001	NS	72.8
Drumstick weight g	0.044	NS	0.008	NS	53.3
Thigh weight g	0.007	0.001	NS	NS	65.9
Relative breast weight	0.003	0.003	NS	NS	51.3
Liver weight	0.020	0.014	NS	NS	64.4
Spleen weight	0.04	NS	0.006	NS	57.3

Discussion

To obtain production efficiency in broiler chicken farming the farmer should achieve ADG of ≥50 g, FCR ≤1.85, PER ≥ 3:1 and slaughter live weight of 1.5-2 kg at 35 days when chicken are reared under optimal management and adequate nutrition [7].

Trial/ experimental Diets

The analyzed crude protein content for BSF larvae meal was 48.5%, a figure almost equal to 47% reported by Sumbule but higher than 39.4% reported by Kirimi *et al.* [20], 43.9% reported by Onsongo *et al.* [23] and 36.9% obtained by De Marco *et al.* [11]. It was however lower than 52.3% reported by Tschirner & Simon [26] and 51.17% reported by Palma *et al* (2020). Similarly, ether extract content of 23.5% in meal was lower than 44.84%, 32.5% and 34.3% reported by Kirimi *et al.* [20], Shumo *et al.* [25] and de Souza Vilela *et al.* [12] respectively. The larvae of black soldier fly stores large quantities of fat as an energy source to carry through pupation and adult stage. It is well noted that the fat content of the BSFL meal in this study is low accompanied by high CP content as illustrated by Tschirner & Simon [26] Jucker *et al.* [19], Njoki *et al.* (unpublished). The amount of CP content in the BSF larvae meal can be modulated to a higher level by the age at harvest and defatting using different methods. It can also be achieved by optimal proximate composition of CP, fat, CF and NFC in the rearing substrate (Njoki *et al*-unpublished).

During diet formulation, we did not encounter major restrictions in increasing the larvae meal up to 30% inclusion level because the fat content was relatively low in the larvae meal utilized for the experiment. It was also easy to formulate iso-nitrogenous diet because the CP content of BSF larvae meal was high; 48.5%. However, the established crude fibre (CF) content requirement for broilers during the finishing phase was slightly exceeded and ranged between 6.0%-8.9% compared to the recommended range of 2%-5% (NRC, 1994). The fat and fibre content of the trial diets increased numerically as the inclusion level of the BSF larvae meal increased which is in agreement with Kirimi *et al.* [20] and de Souza Vilela [13]. The range of fat content in the trial diets was 4.0-9.2% and was within the recommended range of 6.84-12.11 except the control diet which was soy bean meal based (NRC, 1994). The CF content was higher than the 2-3% usually contained in the broiler commercial diets. High levels of crude fibre in the diet decrease energy density thereby increasing feed intake (15; 5). However, CF is also a functional component of normal digestive organ function and moderate dietary fiber could promote the development of digestive organs, increase digestive enzyme activity and nutrient digestibility, improve health status and enhance growth performance in poultry.

In addition, Zhang *et al.* [29] documented that 7-9% CF content to be optimal and may promote growth performance by improving the nutrient digestibility, immunity and intestinal morphology of broilers from day 22 to 42.

Feed Intake, Growth Rate, Feed Efficiency and Protein Efficiency Ratio

Feed Intake

There was a slight numerical but non statistical increment in the total feed and average daily feed intake for the chicken fed 15% and 22.5 % inclusion level of BSF larvae meal compared to the control and 30% BSF larvae meal -based diet. The highest intake was 4920 g and the lowest was 4659 g for 15% and 30% BSF larvae meal respectively. This is in contrast with Kirimi *et al.* [20] who reported a noticeable decrease in the average daily feed intake of birds with successive increases in larvae meal in the diets. This may be attributed to increased fat content as a result of increased levels of BSF larvae meal with high fat content of 44.84% of the meal utilized in the said study which increased dietary energy density, thereby decreasing feed intake [20]. In the current study the fat content in the diets ranged between 4.9-9.2% which was slightly higher than 3-4% used in the commercial broiler finisher diets. The average daily feed intake (ADFI) was not significantly different ($P<0.5$) and ranged between 168-175.71g. This was slightly higher than 150.70g which was reported by Onsongo [23] at 15% inclusion level and Kirimi *et al.* [20] at 18% and 25% inclusion level respectively. This disparity in ADFI could be contributed by high live weight of the broiler chicken in the present study because heavier birds will take more feed compared to lighter ones for maintenance requirement. The hypothesis of chitin being the cause of reduced feed intake with increased level of BSF larvae meal was not observed in the current study and should be interrogated further since insect form the natural feed base for chicken on free range production system. The observed tolerance for BSF larvae fat may be because insects form part of the natural instinctual diet of chickens, which might suggest that the young chicks are not as negatively affected by BSF larvae fat as they are by other fat sources. Future studies would need to compare the tolerance fat digestibility from other sources with BSF larvae fat.

Final Body Weight

The present study showed that the use of black soldier fly larvae meal in feeds increased the final weight and weight gain of the broiler chickens significantly ($p<0.05$). This is in line with Schiavone *et al.* [30] who documented an increase in the growth rate of broiler chickens given black soldier fly larvae meal. It has also been documented that body weights of chickens fed standard feed supplemented with 8% whole de-frozen larvae were higher than those of control chickens. These values of the final live weight of 3064g, 3331 and 3499g at inclusion level of 0, 7.5 and 15% is higher than 3071, 3182, 3006 and 3033 grams reported by Onsongo (2018) at 49 days at the larvae inclusion level of 0, 5, 10 and 15%. At the age of 35 days the chicken weighed 1983 to 2160 g which is also comparable to 2082g reported by Uushona [27] at the same age and at 15% inclusion level. Diets containing 30% soy bean meal and 30% BSF larvae meal had the lowest live weight statistically despite all the diets being isocaloric and isonitrogenous.

Further, there was a quadratic response in final weight with consecutive increase of BSF larvae meal as illustrated in Figure 1. The weight increased to a maximum of 3499 grams attained at 15% inclusion level which was not statistically different from 3331.25g and 3382.33 g attained at 7.5% and 22.5% inclusion level respectively.

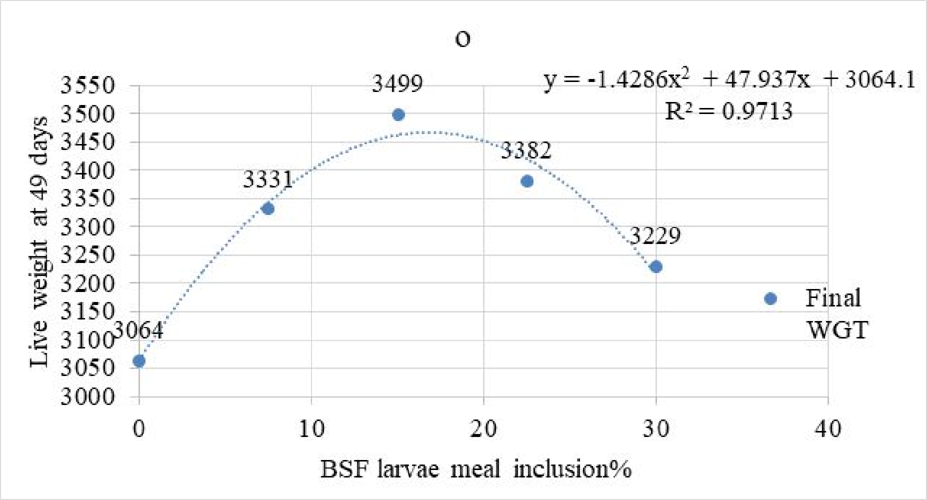

Figure 1: Final body weight of broilers at 49 days old

These results are consistent with previous studies, and reconfirm that dose-response effect of including BSF larvae meal in chicken diets was observed, with high inclusion levels negatively impacting on poultry performance [22]. The broilers which were fed diets containing a combination of soy and BSF larvae meals resulted to higher final weight than those fed either soy or BSF larvae meal as the sole source of CP. The enhanced weight in the current study could be due to availability of all the nutrients required for muscle deposition when soy and BSF larvae meals are combined in the diet while on the other hand the requirement was not attained by either of the protein source when utilized as the sole source. The same trend was observed in the total weight gain and average daily gain performance values.

Poor growth performance in the control diet (30% soy bean meal based) despite similar crude protein levels across the diets implied there was inadequate supply of nutrients to the broilers. The 30% soybean meal-based diet was probably deficient in cystine which was not supplemented as was the case for lysine and methionine amino acids (20). Soybean also contains proteinase inhibitors which may reduce the availability of amino acids leading to reduced deposition of protein for muscle development.

The depressed final weight when BSF larvae meal was utilized as the sole source of CP could be partially, attributed to imbalance in amino acid uptake. It has been established that BSF larvae meal is deficient in arginine amino acid, and this deficiency may have increased as the levels of inclusion increased [17]. Although the levels of the two amino acids were not established in the current study the supplementation of lysine AA across the diet may have negatively impacted the arginine to lysine ratio to levels that were below the NRC recommendations of 0.9 to 1.18 in diets containing 30% BSF larvae meal and the imbalance could have been exacerbated by supplementation with synthetic lysine. Therefore, an imbalance in the arginine: lysine ratio in diets containing high levels of BSF larvae meal may in part, explain the poor performance of the broilers observed in this and other studies. In line with this, substitution of soy bean meal with 50% BSF larvae meal in diets fortified with the AAs lysine, methionine, arginine, and valine was shown to improve the performance of broiler chickens compared to diets containing 50% BSF larvae meal and a basic level of AA supplementation [28]. This is in agreement with Fruci *et al.* [18] indicating that broilers fed high levels of BSF larvae meal were not receiving sufficient AAs including arginine in their diets.

The other cause of depressed final live weight with progressive inclusion of BSF larvae meal in the diets could be the presence of high content of chitin which was quantified as crude fibre in the present study which was 16.2% on DM basis. Chitin is the main component of the arthropod exoskeleton which is embedded in a matrix with protein, lipid and minerals. During the prepupae stage (instar 5), which is recommended for feeding purpose chitin ranges between 4.3-19.1% [14].

Several authors have documented on how high chitin content is the causative effect of depressive growth. The first effect is reduced protein digestibility due to impaired accessibility of digestive enzymes to these nutrients in the matrix constellation [8]. This has been confirmed by Marono *et al.* [22] in *in vitro* crude protein digestibility which showed negative correlation between the chitin content of black soldier fly meal. The reduced CP digestibility result to decreased final live weight [1]. The other cause of depressed live weight at 30% BSF larvae meal inclusion may be due to decreased digestion and absorption of nutrients which is directly affected by morphology of the small intestine, especially the crypts and villi of the absorptive epithelium. Although intestinal morphology analysis was not conducted in the current study, shorter villi, deeper crypts and reduced villus height to crypt depth were observed in broilers fed diets containing 15% BSF larvae meal compared to 5% and 10% inclusion level [10]. Cutrignelli *et al.* [9] reported similar findings in jejunum and ileum of laying hens fed diets with BSF larvae meal as complete replacement of soybean meal.

Short villi and deep crypts are indicative of negative gut development, reduced surface area for the absorption of nutrients and are indicators of a decreased villus function. In addition, larger crypts may be related to an increase in cell turn-over, which also involves an increase in energy requirement for gut maintenance, with subsequent utilization of the nutrients especially protein for rejuvenation of the villus. The consequential undigested protein may increase the quantity of undigested AAs, with a subsequent enhanced proteolytic fermentation by the resident microbiota and formation of toxic compounds, such as amines, ammonia, skatole or indoles.

In addition, chitin is an insoluble polymer and binds water by surface tension or hydrogen bonds hydrogen bonds in the pores of its matrix. As such, a poultry diet containing a higher amount of chitin result in feed bulking hence increase the transit time of digesta and passage of nutrients in the lower part of GIT. This decreases digestion due to its limited accessibility by the digestive enzymes leading to reduced bio-availability of the nutrients to the animal being fed. Research studies document reduced digestive tract transit time of almond shell which contained low hydration capacity compared to lignocellulose, fine and coarse ground straw diets with high hydration capacity resulting to reduced FCR and ADG.

However, low chitin content of between 1.36% to 2.42% has been shown to have beneficial effects on growth performance and no adverse effect on digestibility. Studies have demonstrated that chitin and its derivatives have an enhancing effect on the responses of innate and adaptive immune system, including recruitment and activation of innate immune cells and generation of cytokine and chemokine. Chitin has antibacterial, antifungal and antiviral activity when it is utilized as a feed supplement [24]. In addition, chitosan which is a derivative of chitin promotes growth in poultry when used as a feed supplement by promoting antioxidant status, immune response and improved intestinal development.

The chitin content of BSF larvae meal appears to vary and has been estimated to range from 2.8 to 7.29% [22]. The stage of development is one of the factors that affect the amount of chitin content in BSF larvae. Chitin is present in all the life stages of BSF and that the relative chitin content increases between the late larvae and the pupae stage, followed by a decrease during the adult stage [14]. The other factor is the quantification method whereby direct determination; acid detergent fibre (ADF) with and without amino acid correction, crude fibre (CF) and neutral detergent fibre (NDF) results in higher chitin contents than indirect determination; spectrophotometry, and ultra-performance liquid chromatography [14]. Direct determination can overestimate the chitin content in insects, as the treatment conditions might not be suitable to fully remove all other nutritional components such as amino acids. Crude fibre present in the GIT of the larvae originating from the rearing substrate can result in an overestimation of chitin content and fasting of the larvae before harvesting is recommended to correct the anomaly [14].

In conclusion, the current study has demonstrated that the BSF larvae could enhance live weight of broilers and could be utilized as partial source of protein with inclusion of 15% and 22.5% of the total diet resulting in superior performance as illustrated in Figure 2.

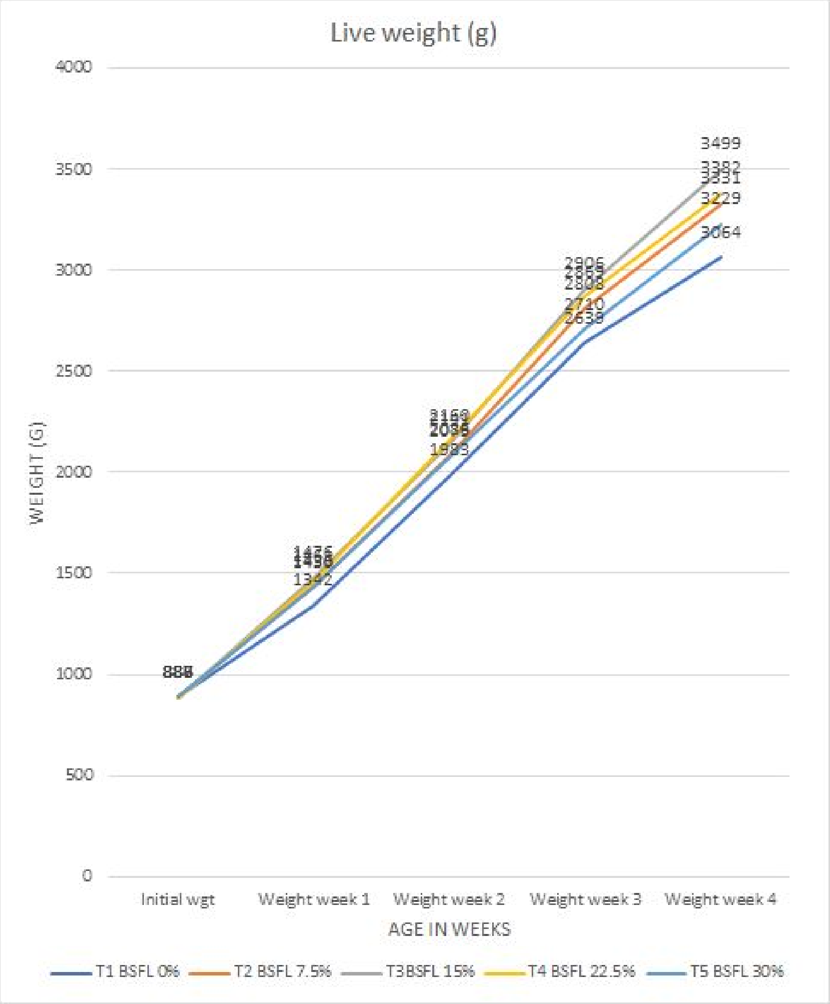

Figure 2: Weekly live weight of broilers fed on diets containing varied levels of BSF larvae meal

Total and Average Weight Gain

There was a quadratic dose response effect on the total live body weight gain (TLWG) and average weight gain (ADG) as the BSF larvae meal level increased. The (TLWG) and ADG ranged between 2180g to 2587g and 77.00g to 92.42g respectively and was significant (P<0.05) between the control and the BSF larvae meal diets. The TLWG is illustrated in Figure 3.

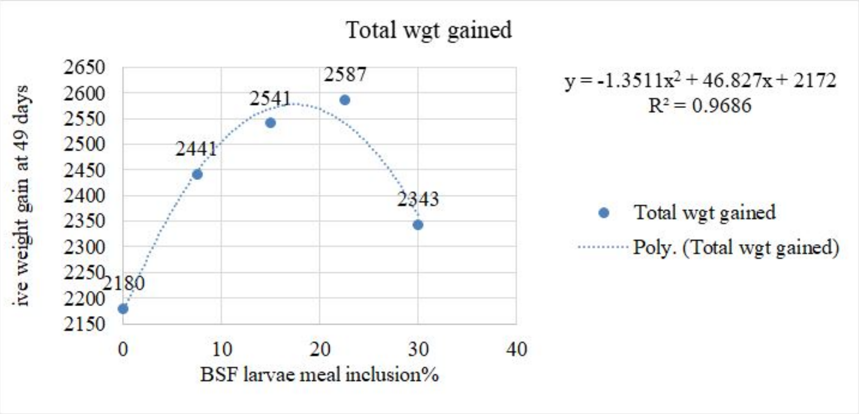

Figure 3: Total weight gain of broiler chicken fed on diets containing varied levels of BSF larvae meal.

The 22.5% inclusion level had the highest TLWG and ADG while the soy meal diet had the lowest and was a reflection of the final live weight. The ADG value in this study attained the industry recommendation of ≥50g for economic feasibility of broiler production. The ADG in the current study was comparable to 79.60g documented by Onsongo [23] at 15% inclusion level at 49 days but higher than 65.4 g by Kirimi *et al.* (2023) at the inclusion level of 25% at slaughter age of 42 days. It is also higher than 65.8g by Uushona. [27] and 60.20 by Cockcroft (2018). The disparity in the ADG in the present study could be the age of termination of the study (49 days) compared to 35 and 32 days by Uushona [27] and Cockcroft [9] respectively. These additional days of production led to additional feed intake which increased exponentially, could explain the inflated ADG [9].

Feed Conversion Ratio

Feed is typically the most expensive cost in broiler production and is the primary tool by which a flock performance is evaluated. Feed conversion ratio (FCR) measures the relationship between the amount of feed consumed and the weight gained by the animal. This ratio effectively measures an animal's efficiency of converting feed into the desired product which in the case of broiler chickens is meat, or body mass. When animals receive diets that meet their nutritional requirements, they convert feed more efficiently, which lowers the ratio. Lower FCR value is an indication of higher efficiency hence better profitability. Efficient feed conversion means that farmers will reduce feed use, resulting in lower production costs. Conversely, a higher FCR can indicate inefficiencies in the production process which are related to low quality of the feed, poor health of the flock and poor management practices.

The FCR posed a quadratic dose response in the present study as illustrated in Figure 3. It was significantly ($P<0.5$) different between the BSF larvae meal diets and the control diet (soy 30%) and it ranged between 1.88 to 2.18. The soybean -based diet scored the highest FCR value of 2.18 at 30% inclusion level while the 15% BSF larvae meal inclusion level scored 1.88 over the total trial period. The BSF larvae meal diets had similar linear FCR score statistically. The recommended industry standard for FCR value is ≤1.85 for the efficient production of broiler chickens [7]. Therefore, the trial diets slightly fell below this expectation with the soybean meal diet having the lowest performance of 2.18 while the 15% BSF larvae meal diet scored 1.88 which is close to the industry requirement of ≤1.85. The BSF larvae meal diets had a good FCR score because it was below 2.0 and this indicated that the digestibility was good and could support good growth performance for broiler chicken. The FCR in this study is comparable to 1.9-2.0 documented by Onsongo. (2018), but higher than 1.41 reported by Cockroft. [9] and 1.5 by Uushona [27]. However, it was lower than 2.04 documented by Kirimi *et al.* [20].

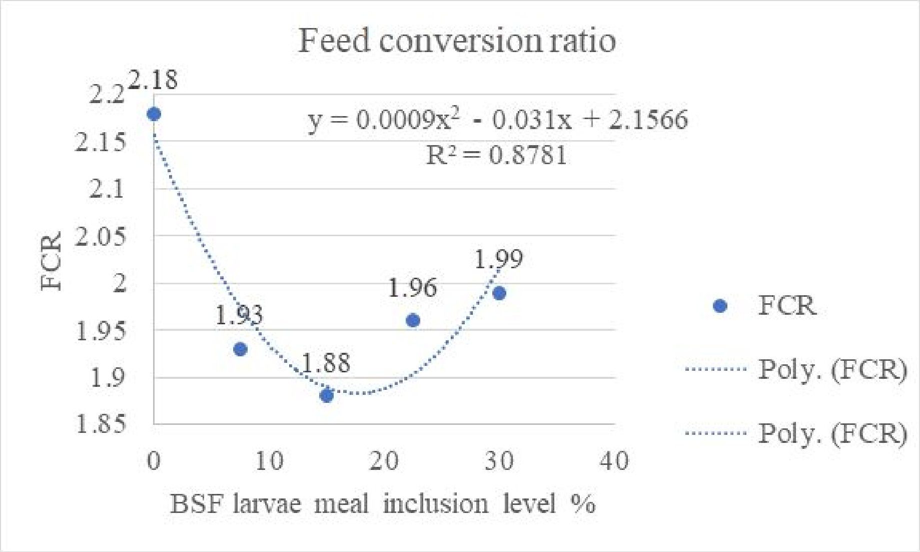

Figure 3: Feed conversion ratio of broilers chicken fed diets containing varied levels of BSF larvae meal

All the BSF larvae-based diets had a minor deviation from the industry standard of which could be nutritional related because the diets contained comparatively high fibre content of 6.7-8.9 % content which is above 2-5% in commercial broiler finisher feeds and more so attributed to presence of chitin which may have reduced digestibility. The BSF larvae meal may have posed amino acid imbalance because it has inadequate amount of arginine. The FCR numerically increased as the larvae inclusion increased which resonates with increased fibre content and arginine deficiency.

Another causative factor of reduced feed efficiency could be the advanced age of the broilers coupled with high live weight at the termination of the experiment. As birds advance in age, they have greater body mass to maintain hence more energy and amino acids are partitioned towards maintenance nutritional requirement. Less nutrients are availed for body mass accretion with ultimate reduced feed efficiency. An increase in feed intake to take care of more body accretion is limited by the gut capacity in a situation where the chicken is offered high nutrient density feed. Nikola *et al*, (25) had documented this whereby broilers recorded FCR value of 1.3-1.4, 1.7-1.9, 2.3-3.0 and 1.8-2.1 when calculated at 1-14 days, 15-35 days, 36-42 days and entire period of 42 days. In this study the FCR was 1.79, 1.81, 1.84, 1.81 and 1.85 for 0, 7.5, 15, 22.5 and 30% inclusion level respectively when calculated for 21-35 days. This is the age recommended by the industry for slaughter and in this study the chicken were not only within the recommended FCR but also had attained the recommended live weight of 1500-2000g. The FCR at 35 days was not significantly (P<0.05) different across all the diets and the weight ranged between 1983-2160 g.

In this study, dietary inclusion of BSF larvae meal up to 30% in feed had a significantly better (p<0.05) FCR value compared to the control diet. This indicated that feeding black soldier fly larvae meal had no detrimental effect on the digestibility in the chickens. This is a positive finding in support of the viability of use of BSF larvae meal poultry feed production. The arginine deficiency could be corrected by incorporation of a synthetic source.

Protein Efficiency Ratio

Protein efficiency ratio (PER) is a method of assessing the quality of protein in the feed consumed by an animal and provides a reliable measure of the value of dietary protein. It explains the protein utilization by the animal and allows for quantification of the carry-over of dietary protein to body mass of the livestock. A low PER value of <1.5 is an indicator of low protein quality in the diet and its utilization by the animals [27]. In the current study, PER presented a significant quadratic dose response (P<0.05) as depicted in Figures 4.

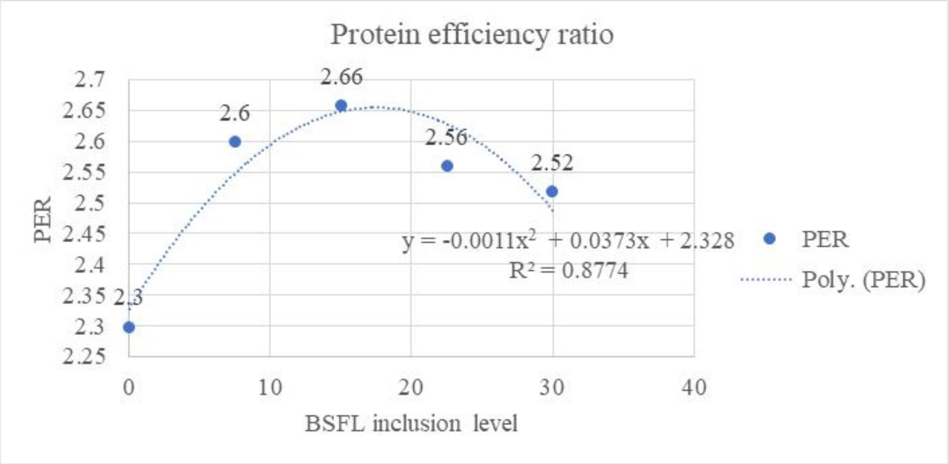

Figure 4: The effect of BSFL inclusion level on protein efficiency ratio

The PER means were 2.3, 2.6, 2.66, 2.56 and 2.52 for control (30% soybean meal), 7.5%, 15%, 22.5% and 30% BSF larvae meal inclusion levels respectively. The industry optimum PER recommendation is 3:1 for broilers. The 30% soybean meal-based diet scored lowest, 30% larvae meal was intermediate while 7.5, 15 and 22.5% larvae meal diets which were similar statistically scored the highest PER values of 2.6, 2.66 and 2.56 respectively. These diets were blends between soybean meal and BSFL meal at various inclusion levels indicating that better PER was a result of better AA balance than when either of the two crude protein source was utilized singularly for formulating the feeding diets. These values were better than 2.5 reported by Uushona [27] at 15% inclusion level but lower than 3.25 reported by Cockcroft [9] at the same inclusion level. However, PER is evaluated on the amount of protein consumed and the trial diets by Cockcroft had a higher protein content of 32%, 19% and 23% for starter, grower and finisher diet respectively which contributed to the disparity because more crude protein was consumed by the said study.

In this study even though the BSF larvae meal inclusion levels affected PER the values obtained indicated that dietary protein was efficiently utilized with a minimum PER of 2.52 as indicated in Table 3. The treatment difference in the PER may be as result of unbalanced amino acid profile in the soy diet while reduced digestibility may an additional cause in the 30% larvae diet coupled with low arginine content and imbalance in arginine: lysine ratio. In an animal diet, amino acid balance is more essential than the protein percentage as the amino acids regulate growth and determine the protein quality of the feed source [6]. The amino acid values and digestibility were not determined in this study and can only rely on literature reports. The limiting amino acids for poultry are lysine for muscle growth and methionine for feather growth. However, the two were supplemented with synthetic source but an imbalance in the arginine: lysine ratio in diets containing high levels of larvae meal has been established [17]. This could in part, explain the lower PER score of the broilers fed 30% BSF larvae meal observed in this and other studies. In line with this, substitution of soy meal with 50% BSF larvae meal in diets fortified with the AAs lysine, methionine, arginine, and valine was shown to improve the performance of broiler chickens compared to diets containing 50% BSF larvae meal and a basic level of AA supplementation [28]. This is an indication that broilers fed high levels of larvae meal were not receiving sufficient AAs including arginine, in their diets and it is imperative that these amino acids be given extra consideration when formulating broiler chicken diets whilst studying suitable protein sources. If the limiting amino acids can be supplied in the appropriate concentrations, the protein utilization and further protein efficiency can be assessed in an objective manner without confounding results.

Carcass Component Yield

Consumers are becoming more aware of meat quality and are also concerned with the ‘value for money’ concept, which only applies to heavier whole carcasses. Heavier carcasses have higher protein deposition than smaller carcasses [5]. Heavier carcasses are meatier in comparison to smaller carcasses, which have higher bone tissue yield. The dressing percentage of a carcass determines the amount of tissue that can be sold in exchange for money whilst the remainder of the carcass is considered as waste. In addition, some consumers prefer to choose from the various portions of the carcass, namely: wings, breasts, drumstick or thighs as opposed to buying whole carcasses being offered for sale in the marketplace. These carcass portions have varying degree of meat available on them and are sold at different prices. It is therefore, important for researchers to test the effects of different feed ingredient and their respective inclusion level on the various portions yield.

Table 4 as earlier presented summarizes the influence of treatment on portion yields and dressing percentage. Dressing percentage gives an indication of muscle, fat and bone growth as well as visceral growth at slaughter time. The dressing percentage is lower when the mass of the visceral organs and the fat percentage is higher. Therefore, these components are inversely related. Higher dressing percentage is advantageous as more meat is yielded from each chicken produced. In the current study, live weight, warm carcass weight and cold carcass weight responded quadratically to increased BSF larvae meal level ($P<0.05$) where 22.5% inclusion resulted in the highest weight. The control diet (30% soybean meal- based) had the lowest while the 7.5%, 15% and 30% were intermediate. The relative dressing percentage was not affected significantly but had a positive linear numerical trend. Uushona [27] found no significant difference in the live weight, cold carcass weight and dressing percentage parameters at the inclusion level of 5, 10 and 15% which is in agreement with the current study. The significant findings in the current study for live weight, warm carcass weight and cold carcass weight results highlighted the treatment difference impacting positively on growth and increased muscle deposition compared with those of the control treatment. Kirimi *et al.* [20] observed the same trend in dressed weight with soybean meal diet and 25% BSF larvae meal inclusion level recording the lowest weight while 12.5 and 18% inclusion resulted in the highest dressed weight. Anankware *et al.* [3] recorded best carcass weight at the inclusion level of 13% and 18%.

The various carcass components are marketed at varying prices and this makes some components attain higher economic importance than others for a processor. The breast component has the highest proportion of meat to bone and it sells at the highest price to the consumers. In the current study, no significant differences were found for the breast portion (P>0.05). This is an indication that all the trial diets contained adequate amount of methionine amino acid which is a structural component of protein in chicken. A study by Zhai and others demonstrated that methionine supplementation in broiler chicken is associated with enhanced growth of breast muscles. On the contrary, significant differences between treatments when comparing the thigh and drumstick portions in terms of weight. The 22.5% BSF larvae meal-based diet drumstick weight was significantly higher than that of the other trial diets. The thigh mass resulting from 30% BSF larvae meal diet was significantly higher than that of the control, as well as the other diets and posted a positive significant (P<0.05) linear trend with increased levels of BSF larvae meal level. The BSF larvae meal -based diets had significantly better PER than the soybean based- diet. The PER could directly influence the thigh and drumstick weight as these are major muscles in the birds' body and therefore a higher protein efficiency will result in higher protein deposition and ultimately bigger and heavier muscles. This observation was in line with that of Schiavone *et al.* (2018) who observed increased thigh weight at 0, 5%, 10% and 15% inclusion level of BSF larvae meal in broiler diets. The BSF larvae meal-based diets had significant PER difference which was expressed in the mass accretion in the thigh and drumstick carcass portion. Uushona (27) found no significant differences in portion yields in a study involving 5%, 10% and 15% inclusion levels of full fat BSF larvae meal.

The portion weight was calculated as a percentage to dressed carcass weight. The thigh and drumstick portions showed no significant difference when compared as a percentage of dressed carcass weight basis. However, there was linear significant decline difference demonstrated on the relative breast weight. This is a translation from no significance difference of the breast portion across the diets yet with a significant high relative weight of soybean meal -based diets compared with the BSF larvae meal-based diets. The soybean meal diet had the highest breast relative value of 43.4% while the 30% BSF larvae meal diet had the lowest relative score of 37.1%. The 7.5%, 15% and 22.5% BSF larvae meal- based diets had intermediate relative breast weight.

Internal Organs

Supply of adequate nutrients is important for optimal health and immune responsiveness in chicken farming. Feed may alter or affect the structural integrity of organs depending upon its nutritional composition and feed granule size. Poor development of organs in chicken has been associated with arginine deficiency. It also negatively affects the development of lymphoid organs which are essential for immune responses of birds. The lymphoid organs in avian are the spleen and bursa fabricious which ensure that pathogens do not invade the host, resist infections and maintain productivity during infectious attacks (9). The bursa is the only lymphoid organ that acts as both a primary and secondary lymphoid organ in avian species. The B-cells produced by the bursa are responsible for anti-body production and fighting antigens present in the birds' bodies. Changes to organ weight is indicative of further health concerns, and therefore can aid in deducing information on the impact of BSF larvae meal on broiler chickens [17]. It is therefore important to evaluate the measurement of the bursa mass and the spleen mass, as well as the ratio between these two organs in order to determine the level of infectious activity the bird has been exposed to throughout its lifetime.

The organs weight and relative weight to the live weight are presented in Table 5. There was no significant (P>0.05) effect of BSF larvae meal inclusion on the weight of the heart, bursa and spleen: bursa ratio and the same applied to the relative weight of all the organs under investigation. On the contrary, the inclusion of the larvae meal increased the weight of the liver, spleen and gizzard significantly (P<0.05). The liver presented a linear increase in weight while the spleen and the gizzard presented a quadratic response with 22.5% larvae meal inclusion level recording the highest weight and then a decrease at 30% level. The weight at 0% and 30% inclusion recorded statistically similar spleen and gizzard weight. The linear increase of the liver weight in this study was in agreement with the finding of Facey *et al.* [17] who also observed a linear increase as the level of BSF larvae meal increased in the trial diets. The increase in gizzard weight may have been due to the bulkiness of the diets containing BSF larvae meal because they contained more crude fibre content with subsequent increased levels of the larvae meal. The results are consistent with Kirimi [20] who reported an increase in gizzard weight. However, in the current study the increase in the gizzard weight was correlated to the larger live and carcass weight of the corresponding treatment. The corresponding decreased gizzard weight in the diet containing 30% BSF larvae meal despite a higher crude fibre content may imply that the chitin which forms a significant portion of fibre in insect meals is not a true fibre and therefore does not exact any extra pressure on the work of the gizzard ultimately necessitating more development and function of this organ. The heart weight was not significantly (P>0.05) different which implied that the diets did not exact excess work on the heart despite increase in live weight of the chicken involved in the study.

Although the spleen weight was affected by larvae meal inclusion level, it could not be related on immune status indication because the relative weight to live weight was not affected and the same applied to the ratio of spleen to bursa. A decrease of bursa weight relative to live body weight was a possible indicator of potential increase in immunosuppression of the immune system which was not the case in the present study. This allows us to assume relatively similar immunity across the trial diets with the conclusion that the BSF larvae was safe to use in broiler diets in relation to their organs' development and overall health performance.

Gastrointestinal Tract pH

The modern broiler chickens have been selected for fast growth, any alternative feed source must support normal bird growth and should not cause any adverse effects on the chicken's health. The gastrointestinal tract (GIT) pH of is one of the factors considered important for health status of chicken because it impacts on the growth and maintenance of the GIT microbial community [9, 21]. The pH in the various segments of the GIT affect the specific digestion and absorption of nutrients which occurs in that region. The pH of feed is typically close to neutral, the crop of the chicken is mildly acidic, the proventriculus and gizzard are acidic while the intestine is mildly acidic at the proximal end, becoming mildly alkaline to neutral toward the distal part. Mabelebele [21] documented 6.08, 4.65 and 3.47 as the pH values for the crop, provetriculus and gizzard for Ross 308 broiler breed. The normal pH ranges of the duodenum, jejunum and ileum are 5.5-6.2, 5.8-6.9 and 6.3-8.0 respectively in healthy chicken.

The results of the pH values of the segments under evaluation in the present study are

Presented in Table 6. They ranged between 3.73-5.23, 3.2-4.64, 2.08-3.31, 4.58-5.05, 4.93-5.51 and 5.67-6.2 for crop, proventriculus, gizzard, duodenum, jejunum and ileum respectively. The trial diets had significant (P<0.05) effect on the gizzard pH values only while the value for the other GIT segments were not affected (P<0.05). The pH values for the crop, proventriculus and gizzard were below than the respective normal values of 6.0, 4.6 and 3.7 [21]. Equally the pH values for duodenum, jejunum and ileum were below the respective normal values of 5.5-6.2, 5.8-6.9 and 6.3-8.0. However, the effect was not related to the BSF larvae meal inclusion level since even the control diet was not within the normal range.

It is worth noting that the pH values for the duodenum, jejunum and ileum values tended to acidic and lower than 6.0-6.3, 6.3-6.4 and 6.8-6.9 documented by Uushona [27] but close to 5.8-6, 5.8-6 and 5.8-6.7 documented by Cockcroft (2018). Maize was the main source of energy but varying inclusion levels in the three studies; 46-52% [27], 65-61% [9] and 65% in the current study. The comparative low pH in the current study could be due to provision of high level of carbohydrate to the gut microbiome from maize which was included at 65% across all the diets.

The GIT of chickens is enriched with complex microbial communities including bacteria, fungi, archaea, protozoa, and viruses but are dominated by bacteria. The host accommodates and forms a symbiotic relationship with the resident bacteria. In poultry, microbiota fermentation of carbohydrate mainly occurs in crop and caecum where bacteria are abundantly populated. The crop in particular harbors a large population of lactobacilli. These bacteria partially ferment the carbohydrates in the feed and produce lactic acid which reduces the pH of the crop environment. The crop and ileum are the main lactic acid-producing repertoires, owing to the presence of a high concentration of *Lactobacillus spp*. This could be the main cause of low pH in the crop in the current study. Therefore, the acidic crop environment due to presence of lactic acid may have carried through into the provetriculus and gizzard further reducing pH of the two segments which are highly acidic in nature. The gizzard also has an acidic environment but has a substantial population of lactobacilli which mainly originate from the crop.

Feed ingredients differentially affect the bacterial communities and the production of metabolites depending on their particle size, type, and chemical properties and that corn-soybean meal-based diet increases the concentration of *Lactobacillus spp*. and production of short chain fatty acids (SCFAs) in duodenum, jejunum, and ileum in broilers of all age groups. The SCFA are a source of energy for the host and are rapidly absorbed by the intestinal epithelial cells and regulate a number of cellular functions including gene expression, chemotaxis, differentiation and apoptosis [9]. They also reduce the pH at the site of production and expedite the nutrient absorption.

Conclusions

The followings conclusions made;

- BSFL meal can be included up to 22.5 % in broiler finisher diets because a higher level of inclusion results in poorer growth performance.
- Feed conversion ratio and protein efficiency ratio was optimal at about 15% inclusion level in the diet.

Recommendations

- The nutrition requirement of the BSF larvae should be adhered in relation to the rearing substrate so as to produce larvae meal with high CP contents (≥45%) and relatively low fat contents (≤25%) and ultimately allow high inclusion level in the broiler diet.
- The level of inclusion of BSFL to replace soybean meal in broiler finisher diets should not exceed 22.5% for optimal growth performance.

Further Research

- Feeding trials to be conducted using BSFL meal of different CP content to assess the effect of inclusion level on broiler response
- Study to determine the effect of different arginine: lysine and arginine: valine on performance of broilers fed on BSFL-based diets

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