Egyptian J. Nutrition and Feeds (2024), 27 (3):291-301

EVALUATION OF DEFATTED BLACK SOLDIER FLY LARVAE SUPPLEMENTATION IN GROWING BUFFALO CALVES' DIETS

A.A. Marwan[1]; Eman, I. Saddick[2] and U.A. Nayel[2]

[1]*Department of Animal Production, Faculty of Agriculture, Ain Shams University, Egypt*

[2]*Department of Animal Production, Faculty of Agriculture, Menoufia University, Egypt*

(Received 1/10/2024, accepted 10/11/2024)

SUMMARY

This study aimed to evaluate the supplementation of dried defatted black soldier fly larvae (BSFL*; Hermetia illucens*) in the diets of growing buffalo calves. BSFL was prepared to be added in the form of dried powder. Fifteen buffalo calves, 5 months of age and 122.44±12.26 kg of body weight, were divided into three experimental groups in a randomized block design, receiving three experimental diets as follows: diet 1 (D1) represented the basal diet with no supplementation, while diet 2 (D2) and diet 3 (D3) represented the same basal diet supplemented with 5 and 10 kg/ton of BSL, respectively. A growth trial was performed to calculate average daily gain (ADG) for 15-day intervals during 90 days. A digestibility trial was conducted to investigate nutrient digestibility using the acid insoluble ash (AIA) method. Dry matter intake (DMI) and dry matter conversion (kg DM/kg gain) were also observed. Results showed that BSFL supplementation at 10 kg/ton significantly ($p \leq 0.05$) improved all nutrients digestibility, ADG, DM conversion, plasma total protein, and albumin. Supplementation of BSFL at 5 kg/ton significantly increased ADG with significant improvement in the digestibility of ether extract (EE) and nitrogen-free extract (NFE) compared to control group. Supplementation did not significantly affect DMI. All blood plasma biochemical parameters were within the average values of blood characteristics of buffalo calves without any deleterious effect on liver or kidney functions as affected by both levels of BSFL supplementation. BSFL supplementation significantly ($p \leq 0.05$) increased plasma triglycerides in a linear pattern, and conversely, it appeared to have the ability to lower plasma cholesterol concentration.

Keywords*: Black soldier fly, buffalo calves, supplementation, digestibility*

INTRODUCTION

Protein is the most expensive nutrient, contributing to the formulation of ruminants' diets and constantly rising in price. When light and young animals receive protein supplements, their growth is accelerated, and they become heavier and leaner, resulting in a more homogenous flock (Polat, 2000). Fish meal (Hussein and Jordan, 1991) and oilseed meals such as soybean meal, sunflower seed meal, and sesame meal (Wang *et al.,* 2016) are all possibly used as protein supplements for ruminants. Changing the protein source for animals leads to different effects on their productive performance and biochemical blood parameters (Burd *et al.,* 2019; Abo-Donia and Nayel., 2022).

The black soldier fly (BSF; *Hermetia illucens*) is an insect that converts organic wastes into valuable protein source in their bodies (Van Huis, 2013). Thus, it is considered a sustainable solution not only for protein but also for waste management (Odongo *et al.,* 2024). BSF has five phases in their life cycle: egg, larva, pre-pupa, pupa, and adult (Rehman *et al.,* 2023). The most nutrient-rich phases are those of the larvae and pre-pupae (Yu GuoHui *et al.,* 2009; Deshmukh *et al.,* 2024). There are two types of larvae included in the scientific research concerning ruminants' nutrition: full-fat larvae and defatted larvae. According to Lu et al. (2022), crude protein content, on a dry matter basis, varied from 27.5 to 43.9% and from 21.6 to 65.5% in the full-fat and defatted larvae, respectively. The high protein content of black soldier fly larvae (BSFL) makes animal nutritionists use it either as a substitution for soybean meal (Jayanegara *et al.,* 2017a; Kahraman *et al.,* 2023) or as a protein supplementation (Fukuda *et al.,* 2022). Jayanegara *et al.* (2017a) concluded that substitution for soybean meal with BSFL reduced the in vitro nutritive value with a good impact on mitigating methane production. However, Kahraman *et al.* (2023)

recommended the substitution with defatted BSFL up to 40% with a positive effect on in vitro digestibility and rumen fermentation.

Most investigations were carried out using in vitro studies, and only a few in vivo studies were included. To the best of our knowledge, no data was published about the inclusion of BSFL in the buffalo calves' diets. Therefore, this study aimed to evaluate the effect of defatted BSFL as a protein supplement on the performance of growing buffalo calves.

MATERIALS AND METHODS

This study was conducted at the Experimental Research Station belongs to Faculty of Agriculture, Ain Shams University located in Shalakan village, Qalubia Governorate, Egypt.

Ethical considerations:

All the experimental procedures were approved by the Animal care and research Ethics of Ain shams university, agriculture sector committee, with an approval No.4-2024-12.

Larvae and experimental diets formulation:

The 21-day-old defatted larvae of black soldier fly were obtained, in the form of dried powder, from El Nada farm located in Monsha'et El Kanater, Giza Governorate. The larvae were reared on organic waste, then separated from their feed media, and microwave-dried at 65°C in 3 cycles of 7 minutes separated by a brief rest period to allow the steam to escape. After drying, fat was mechanically extracted using a screw-press expeller. Finally, dried and defatted larvae were ground into powder passed through a 1 mm sieve and chemically analyzed.

The basal diet consisted of concentrate feed mixture (CFM) as the concentrate portion while the roughage portion contained berseem and rice straw, with a 40:60% roughage: concentrate ratio, respectively. Three supplementary levels (kg/ton) of dried black soldier fly larvae (BSFL) were included in the CFM as follows: 0 kg for diet 1 (D1), which served as the control diet, 5 kg for diet 2 (D2), and 10 kg for diet 3 (D3). The chemical compositions of feedstuffs, defatted larvae, and experimental diets are shown in table (1).

Table (1): Chemical composition of feedstuffs and experimental diets (% on DM basis).

Items	Ingredients			Supplement	Experimental diets		
	CFM*	Berseem	Rice straw	BSFL**	D1	D2	D3
Dry matter, DM	90.26	16.79	91.21	94.05	68.31	68.34	68.57
Organic matter, OM	92.83	91.34	81.99	87.79	91.30	91.27	91.26
Crude protein, CP	16.39	19.50	3.91	54.12	16.08	16.26	16.45
Crude fiber, CF	19.75	29.03	45.18	12.51	25.08	25.01	24.95
Ether extract, EE	3.06	3.67	1.89	9.11	3.13	3.16	3.19
Nitrogen-free extract, NFE	53.63	39.15	31.00	12.05	47.01	46.86	46.67
Ash	7.17	8.66	18.01	12.21	8.70	8.72	8.74
AIA* **	5.28	4.49	14.01	0.74	5.92	5.89	5.86

D1: Diet 1, BSFL 0 kg/ton; D2: Diet 2, BSFL 5 kg/ton; D3: Diet 3, BSFL 10 kg/ton.
** CFM: Concentrate feed mixture, **BSFL: Black soldier fly larvae, ***AIA: Acid insoluble ash.*
***** The CFM composed of 38% ground maize, 15% soybean meal, 34% wheat bran, 5% rice bran, 3% molasses,1% mineral salts, 2% limestone powder,1% Sodium Bicarbonate and 1% sodium chloride.*

Chemical analysis:

Samples of feedstuffs, defatted larvae, and experimental diets were chemically analyzed according to AOAC (2000).

Animals, management, and experimental design:

Fifteen buffalo calves, with an average age of 5 months and a body weight of 122.44±12.26 kg, were randomly divided into three groups receiving three experimental diets: D1, D2, and D3. Calves were fed according to the nutrient requirements of Kearl (1982) and adapted to the experimental diet for 21 days. Diets were offered daily at 8 a.m. and 4 p.m. clean and fresh water was available to all animals. The experimental design was a randomized block design with 5 replicates for each treatment.

Digestibility trial:

Digestibility trials were carried out for all experimental animals using a grab sample method according to (Schneider and Flatt, 1975), where acid insoluble ash (AIA) was applied to determine the nutrient's digestibility as a natural internal marker. Samples of fresh feces were gathered for consecutive seven days, dried for 24 hours at 65°C in the oven, and mixed before representative samples were obtained and chemically analyzed according to AOAC (2000). The average intake was recorded daily, while the average amount of daily output feces and digestion coefficients of nutrients were calculated using the acid insoluble ash (AIA) method described by Van Keulen and Young (1977).

Feed intake and growth parameters:

Daily feed intake and biweekly body weights were recorded for 90 days to calculate the dry matter intake (DMI), average daily gain (ADG), and dry matter conversion. Weighing calves was performed in the morning before feeding. Calves' total weight gain was calculated as the difference between initial and final body weight. ADG was calculated at biweekly intervals as the weight gain of specific 15 days period divided by 15. The total weight gain was divided by 90 to calculate the total ADG. Dry matter conversion (kg DM/kg gain) was calculated by dividing the dry matter intake (DMI) by the total gain acquired in a certain period.

Blood plasma parameters:

At the end of the study, blood samples were collected in heparinized tubes from each calf through the jugular vein just before morning feeding. The heparinized tubes were centrifuged (2095× g) in a centrifuge at the lab for 15 minutes. The blood plasma (the supernatant) was transported to clean Eppendorf tubes labeled with animal ID and frozen at -20 ºC for later analysis. Plasma samples were analyzed for total protein concentrations, albumin, alanine transaminase (ALT), aspartate transaminase (AST), creatinine, urea, triglycerides, and cholesterol. Albumin was subtracted from total protein to get the globulin content. Analyses were carried out according to the manufacturer's instructions for commercial kits (Stanbio Laboratory, Boerne, TX, USA).

Statistical analysis:

To statistically analyze the experimental data, the General Linear Model of SAS (SAS, 2002) was used as follows:

$$Y_{ij} = \mu + Ti + e_{ij}$$

Where: Y_{ij} = the parameters under analysis. μ = the overall mean. T_i = the treatment effect (i = 1 …and 3). e_{ij} = the random error of means. Differences among treatments were detected using Duncan (1955).

RESULT AND DISCUSSION

Effect of BSFL supplementation on:

Nutrients digestibility:

The effect of BSFL supplementation on nutrient digestibility is presented in table (2). It clearly appears that supplementation of BSFL significantly ($P \leq 0.05$) increased the digestion coefficient of dry matter (DM), organic matter (OM), crude protein (CP), and crude fiber (CF) just at a level of 10 kg/ton. The digestibility values of DM, OM, and CF were 63.8, 69.18, and 62.75%, respectively, for D1, while values were 64.33, 71.86, 63.88%, and 69.79, 76.64, 66.46% for D2 and D3, respectively, with significant differences between D2 and D3. CP digestibility had the highest value (70.85%) in D3, followed by (68.24%) in D2, nonetheless, no significant differences between D2 and D3 were observed. However, BSFL supplementation significantly ($P \leq 0.05$)

increased the digestion coefficient of ether extract (EE) and nitrogen-free extract (NFE) at both levels of supplementation, with no significant differences between the two supplementation levels. The digestibility of EE increased from 80.08% for D1 to 83.14 and 84.22% for D2 and D3, respectively.

Table (2): Effect of BSFL supplementation on the nutrients' digestibility of buffalo calves.

Item (%)	Experimental diets		
	D1	D2	D3
Dry matter (DM)	63.80±1.03[b]	64.33±0.52[b]	69.79±1.56[a]
Organic matter (OM)	69.18±1.25[b]	71.86±1.29[b]	76.64±0.77[a]
Crude protein (CP)	66.10±1.18[b]	68.24±0.84[ab]	70.85±1.02[a]
Crude fiber (CF)	62.75±0.93[b]	63.88±0.79[b]	66.46±0.39[a]
Ether extract (EE)	80.08±0.82[b]	83.14±0.24[a]	84.22±1.14[a]
Nitrogen free extract (NFE)	73.41±1.24[b]	77.45±0.65[a]	79.26±0.36[a]

D1: Diet 1, BSFL 0 kg/ton; D2: Diet 2, BSFL 5 kg/ton; D3: Diet 3, BSFL 10 kg/ton.
[a, b and c] Means of treatments within the same row with different superscript letters are significantly different (P ≤ 0.05).

Kahraman *et al.* (2023) replaced 20 and 40% of soybean meal in dairy cows' diets with defatted BSFL and observed a positive impact on the in vitro digestibility of dry matter (IVDMD) and neutral detergent fiber (NDF) for both substitution levels. They attributed the reason for the reduced ratio of non-fibrous carbohydrate (NFC) to NDF in the diet, which included BSFL; the higher ratio can reduce rumen pH and consequently reduce the ruminal population of fiber-degrading bacteria (Sung *et al.,* 2006), leading to a decrease in NDF digestibility (Pinho *et al.,* 2019). A similar finding regarding increased IVDMD was reported by Phesatcha *et al.* (2022) when soybean meal was replaced with another insect meal known as cricket fly. On the other hand, BSFL meal substitution for soybean meal decreased both IVDMD and in vitro organic matter digestibility (IVOMD) (Jayanegara *et al.*, 2017a). Furthermore, Astuti and Wiryawan (2022) reported that fattening goat on the frass, the residual medium after larvae growth, of BSF decreased the digestibility of DM and OM, arguing the reason for the high content of chitin represented in the frass. Fukuda *et al.* (2022) revealed that supplementation of dried BSFL for beef steers on low-quality roughage did not significantly affect DM, OM, and NDF in vitro digestibility when compared with supplementation of traditional protein sources such as cottonseed and soybean meal. They concluded that BSFL is an effective protein supplement.

In the current study, increased digestibility of CP in the diets supplemented with BSF may be due to the increased CP content in diets supplemented with BSFL (table 1) or/and the high digestibility of BSF protein, since Owens *et al.* (2014) found that diets containing higher CP content increased the activity of ruminal bacteria, especially proteolytic bacteria. Also, Marono *et al.* (2015) revealed that BSF protein had an in vitro digestibility of 66–68% using a two-step enzymatic method.

Currier *et al.* (2004) stated that supplementation of protein sources to ruminants can increase fiber digestibility due to an increased population of cellulolytic bacteria. This might be a probable reason for increased CF digestibility in our study. Jayanegara *et al.* (2017a) noted that supplementation 40% of BSFL caused an elevation in the dietary EE content, suggesting that this elevation was responsible for decreasing fiber digestibility. In the current study, crude fiber digestibility was increased even though the defatted larvae had a 9.1% of EE. This indicates that the supplementation levels did not increase the fat content to a point (table1) where it would have adversely affected the digestion of fiber. Increased EE digestibility, in the current study, might indicate that insect fat had high digestibility.

Dry matter intake (DMI):

The quantities of DM consumed by calves are shown in table (3). At all the periods, the highest DMI was consumed by calves on D3, followed by calves on D2, while the lowest DMI was consumed by calves on D1, except for the period from 46 to 60 days in which the lowest DMI was recorded by calves on D2. The highest DMI achieved by D3 ranged from 4.16 to 5.94 kg/head/day (kg/h/d), with an average of 4.98 kg/h/d. The lowest DMI achieved by D1 ranged from 3.72 to 5.32 kg/h/d with an average of

4.50 kg/h/d. There were not any significant differences among the groups either for a specific period or for the total average.

Table (3): Effect of BSFL supplementation on Dry matter intake (kg/h/d).

Days	Experimental diets		
	D1	D2	D3
0 – 15	3.72± 0.46	3.79± 0.19	4.16± 0.50
16 – 30	4.03± 0.49	4.09± 0.21	4.44± 0.52
31 – 45	4.31± 0.51	4.37± 0.22	4.76± 0.54
46 – 60	4.70± 0.54	4.64± 0.23	5.10± 0.58
61 – 75	4.92± 0.57	5.08± 0.25	5.50± 0.61
76 – 90	5.32± 0.58	5.48± 0.27	5.94± 0.64
Average	4.50	4.58	4.98

D1: Diet 1, BSFL 0 kg/ton; D2: Diet 2, BSFL 5 kg/ton; D3: Diet 3, BSFL 10 kg/ton.

Astuti *et al.* (2019) noted that goat rations containing 15 and 30% cricket meal did not affect DMI. Astuti and Wiryawan (2022) compared a diet containing 30% BSFL frass with commercial concentrate with the same nutritive value for growing goats; they found no significant differences in nutrient intake. However, supplementation of BSFL for beef steers on low-quality forage led to an increase in forage intake without significant differences between BSFL and conventional protein supplements (Fukuda *et al.*, 2022), which indicates that its previous effect on DMI was due to increased protein level. Also, Bohnert *et al.* (2011) reported an increase in DM intake due to protein supplementation either for steers or wethers. Furthermore, Amanlou *et al.* (2017) demonstrated that increasing dietary CP level from 16 to 19% led to an increase in the DMI, nonetheless, no effect was observed when the dietary CP level was over 19%. Unfortunately, only a few published data were available about the effect of BSFL on DMI.

Growth parameters and DM conversion:

The changes in the biweekly body weights and the DM conversion are shown in tables (4) and (5), respectively. From table (4), The initial body weights were 119.33, 119.00, and 129.00 kg for D1, D2, and D3, respectively. The highest final body weight and total gain for 90 days were recorded by calves on D3, being 198.00 and 69.00 kg, respectively

Table (4): Effect of BSFL supplementation on the changes of body weights and daily gain (kg/h/d).

Item	Experimental diets		
	D1	D2	D3
Animal weight			
Initial weight	119.33± 14.62	119.00± 6.65	129.00± 15.52
Final weight	178.66± 19.64	181.66± 9.27	198.00± 21.51
Total gain	59.33± 5.04	62.66± 2.66	69.00± 6.00
Days		**Average daily gain (kg/h/day)**	
0 – 15	0.422±0.05	0.488±0.02	0.644±0.08
16 - 30	0.622±0.05	0.511±0.04	0.622±0.05
31 – 45	0.688±0.05	0.622±0.02	0.711±0.05
46 – 60	0.755±0.06	0.755±0.02	0.755±0.08
61 – 75	0.622±0.05	0.844±0.04	0.888±0.05
76 – 90	0.844±0.04	0.955±0.05	0.977±0.08
Average	0.659^{c}	0.696^{b}	0.766^{a}

D1: Diet 1, BSFL 0 kg/ton; D2: Diet 2, BSFL 5 kg/ton; D3: Diet 3, BSFL 10 kg/ton.
$^{a,\ b\ and\ c}$ Means of treatments within the same row with different superscript letters are significantly different (P ≤ 0.05).

Calves on D1 showed the lowest final body weight and total gain being 178.66 and 59.33 kg, respectively. In the first 45 days, the highest average daily gain (ADG) was achieved by calves on D3 at all the periods, followed by D1 from 16-45 days. During the periods from 46 to 60 days, the ADG was similar for the three experimental groups, being 0.755 kg/h/d. At all the periods from 61-90 days, the highest ADG was recorded for D3, followed by D2. Generally, the total ADG for 90 days was significantly ($P \leq 0.05$) affected by BSFL supplementation, as the calves supplemented with 10 kg/ton had the highest ADG, followed by the calves supplemented with 5 kg/ton, compared to calves receiving no supplementation. The total ADG values were 0.659, 696, and 0.766 for D1, D2, and D3, respectively. Increased average daily gain could be, most likely, due to increased nutrient digestibility in BSFL-supplemented diets (Table 2).

Table (5) shows the efficiency of converting DM to gain. The highest efficiency was achieved by calves receiving the basal diet without supplementation, followed by calves on D3 during the periods from 16-45 days, while the calves receiving D2 recorded the highest efficiency, followed by D3 during the period from 61-75 days. BSFL supplementation significantly ($P \leq 0.05$) improved the total average of DM conversion for 90 days from 6.58 KG DM/kg gain for D1 to 6.28 and 6.09 kg DM/kg gain for D2 and D3, respectively, with significance only between D1 and D3. This is consistent with the results of digestibility (table 2), especially with the convergence of DMI quantities (table 3), as higher digestibility provides more energy and protein available for utilization per kg of DM, leading to more efficient DM conversion.

Table (5): Effect of BSFL supplementation on dry matter conversion (kg DM/ kg gain).

Days	Experimental diets		
	D1	D2	D3
0 – 15	8.52±0.23	7.34±0.59	6.01±0.15
16 – 30	6.02±0.17	7.45±0.27	6.67±0.33
31 – 45	5.84±0.20	6.45±0.13	6.24±0.49
46 – 60	5.73±0.23	5.68±0.13	6.30±0.02
61 – 75	7.52±0.16	5.49±0.06	5.70±0.30
76 – 90	5.86±0.36	5.26±0.17	5.60±0.18
Average	6.58^{a}	6.28^{ab}	6.09^{b}

D1: Diet 1, BSFL 0 kg/ton; D2: Diet 2, BSFL 5 kg/ton; D3: Diet 3, BSFL 10 kg/ton.
$^{a, b \text{ and } c}$ Means of treatments within the same row with different superscript letters are significantly different ($P \leq 0.05$).

Astuti *et al.* (2019) reported that supplementation with cricket meal did not affect goats' daily gain or feed conversion. Zhao *et al.* (2023) demonstrated that as the dietary protein increased, the weight gain increased when ewes were fed on graded levels of protein. Generally, the response of ruminants to inclusion of insects in their diets might depend on the kind of insect, its nutritional composition, level of inclusion, animal species, and diet composition.

Blood plasma parameters:

Table (6) represents the blood plasma constituents of calves fed experimental diets. The total protein concentration was significantly ($P \leq 0.05$) increased from 6.02 g/dl for D1 to 6.26 and 6.50 g/dl for D2 and D3, respectively, as affected by BSFL supplementation. The albumin concentration followed the same pattern of total protein, while the globulin concentration did not show any significant differences among groups. Calves that received D1 had the highest AST concentration, being 40.91 units/L, whereas calves on D3 had the lowest concentration (30.36 units/L), with significance ($P \leq 0.05$) between D1 and D3 and between D2 and D3. The ALT concentration was significantly ($P \leq 0.05$) decreased as the level of supplementation increased, being 37.37, 33.86, and 27.10 units/L for D1, D2, and D3, respectively. Supplementation significantly ($P \leq 0.05$) decreased urea and creatinine either at the level of 5 kg/ton or 10 kg/ton, with no significant differences between both levels of supplementation. The values of urea concentration were 36.27, 24.20, and 25.19, while values of creatinine were 1.56, 1.25, and 1.20 for D1, D2, and D3, respectively. BSFL supplementation significantly ($p \leq 0.05$) elevated blood plasma triglycerides in a linear pattern from the lowest value (52.62 mg/dl) for D1 to 64.76 mg/dl and 80.71 mg/dl for D2 and D3, respectively. As the opposition, the blood plasma cholesterol was significantly ($P \leq 0.05$) decreased as the level of supplementation increased; the highest value (82.70 mg/dl) was recorded by D1, while the lowest value (74.21 mg/dl) was recorded by D3. All parameters were in accordance with the reference range reported by Abd Ellah *et al.* (2013).

Table (6): Plasma constituents of calves supplemented with BSFL.

Item	Experimental diets		
	D1	D2	D3
Total protein (g/dl)	6.02[b]	6.26[ab]	6.50[a]
Albumin (g /dl)	2.33[b]	2.42[ab]	2.60[a]
Globulin (g /dl)	3.69	3.84	3.90
AST (unit /L)	40.91[a]	37.67[a]	30.36[b]
ALT (unit /L)	37.37[a]	33.86[b]	27.10[c]
Urea (mg /dl)	36.27[a]	24.20[b]	25.19[b]
Creatinine (g /dl)	1.56[a]	1.25[b]	1.20[b]
Triglyceride (mg/dl)	52.62[c]	64.76[b]	80.71[a]
Cholesterol (mg/dl)	82.70[a]	78.14[b]	74.21[c]

D1: Diet 1, BSFL 0 kg/ton; D2: Diet 2, BSFL 5 kg/ton; D3: Diet 3, BSFL 10 kg/ton.
[a, b and c] *Means of treatments within the same row with different superscript letters are significantly different ($P \leq 0.05$).*

In this study, elevation in blood plasma total protein and albumin could be attributed to increased CP in the supplemented diets due to larvae protein (table1). Increasing CP levels in the diet through protein supplementation led to an increase in serum albumin (Amanlou *et al.,* 2017). In accordance with our study, Amanlou *et al.* (2017) noted that cows fed diets containing higher CP had lower AST concentration than cows on lower CP content. Concentrations of AST and ALT are often determined to assess how well the liver is functioning. Thus, as our values of AST and ALT are within the normal range, they are good indications that supplementation did not affect liver function.

Increased blood urea was reported due to increased dietary CP levels in most studies (Amanlou *et al.*, 2017; Xia *et al.*, 2018). Runa *et al.* (2022) suggested probable reasons for increased blood plasma urea such as increased urea production, decreased excretion of urea, or a combination of both. Reduction in urea concentration in calves' plasma supplemented with BSFL in the current study might be an indication of lower ruminal-NH3, since Adiwinarti *et al.* (2018) reported that increased rumen degradable protein leads to increased ruminal ammonia and blood urea concentration, which is consistent with the reduction in ruminal-NH3 reported due to the inclusion of BSFL by previous investigators (Jayanegara *et al.*, 2017a; Jayanegara *et al.*, 2017b; Kahraman *et al.*, 2023). Creatinine is a by-product of creatine muscle catabolism, and its concentration rises at the beginning of renal dysfunction (Varga, 2014). In healthy animals with normal water body content and normal renal function, any alteration in skeletal muscle causes a shift in the concentration of plasma creatinine (Wyss and Kaddurah-Daouk, 2000). A reduction in plasma urea concentration in the blood of our calves supplemented with BSFL might indicate lower muscle mass than calves on a control diet.

Increased plasma triglycerides and total cholesterol were often observed by fat supplementation or feeding whole oilseeds (Deng *et al.,* 2018; Saddick *et al.,* 2024). In the current study, increased triglycerides might be attributed to the higher EE digestibility (table 2) as more digestible EE might indicate more fatty acid absorption; hence more triglycerides went into the blood stream. Interestingly, total cholesterol concentration decreased due to supplementation, suggesting that larvae contain one or more substances that lower cholesterol. This might be due to the high presence of calcium in the mineral composition of the larvae. The BSFL are rich in minerals; calcium is the most abundant, ranging from 1.2 to 23.7 g/kg DM (Lu *et al.,* 2022). Hsu and Culley (2006) reported that calcium supplementation significantly decreased serum cholesterol by 30% in rabbits' blood.

CONCLUSION

The larvae of black soldier fly (BSF) could be used as a protein supplement for growing buffalo calves. Supplementation of defatted black soldier fly larvae (BSFL) at 10 kg/ton for growing buffalo calves significantly improved nutrients digestibility, average daily gain (ADG), DM conversion, blood plasma total protein, and albumin without affecting DM intake. All blood biochemical parameters were within the average values of blood characteristics of buffalo calves without any deleterious effect on liver or kidney functions. Larvae of BSF significantly increased plasma triglycerides in a linear pattern, and conversely, seemed to have the ability to lower plasma cholesterol concentration. Further studies are needed to investigate safety, different supplementation levels, and rumen fermentation for more declaration.

REFRENCES

Abd Ellah, M. R., I. Hamed, D. R. Ibrahim and H. Z. Rateb. (2013), 'Reference Values for Hematological and Serum Biochemical Constituents in Late Pregnant Buffaloes', *Assiut Veterinary Medical Journal* Vol. 59, No. 136, pp. 35-46. DOI: 10.21608/avmj.2013.192537

Adiwinarti, R., I. G. S. Budisatria and E. Indarto. (2018), 'Profile of Rumen Fermentation and Blood Urea Nitrogen Concentration of Kacang Goat Fed Total Mixed Ration Vs. Roughage', (Ed.)^(Eds.), IOP Publishing. DOI: 10.1088/1755-1315/119/1/012049

Abo-Donia, F. M. and U. A. Nayel. (2022), Cultivation of housefly larvae (Musca domestica L.) as a biological methods to enrich rice straw and miximise its utilisation.Journal of Insects as Food and Feed, 8 (3), 255-266. doi.org/10.3920/JIFF2021.0055

Amanlou, H., T. A. Farahani and N. E. Farsuni. (2017), 'Effects of Rumen Undegradable Protein Supplementation on Productive Performance and Indicators of Protein and Energy Metabolism in Holstein Fresh Cows', *Journal of dairy science* Vol. 100, No. 5, pp. 3628-3640. doi.org/10.3168/jds.2016-11794

AOAC (2000) Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

Astuti, D. A., A. Anggraeny, L. Khotijah, S. Suharti and A. Jayanegara. (2019), 'Performance, Physiological Status, and Rumen Fermentation Profiles of Pre-and Post-Weaning Goat Kids Fed Cricket Meal as a Protein Source', *Tropical Animal Science Journal* Vol. 42, No. 2, pp. 145-151. https://doi.org/10.5398/tasj.2019.42.2.145

Astuti, D. A. and K. G. Wiryawan. (2022), 'Black Soldier Fly as Feed Ingredient for Ruminants', *Animal bioscience* Vol. 35, No. 2, pp. 356. doi: 10.5713/ab.21.0460

Bohnert, D. W., T. DelCurto, A. A. Clark, M. L. Merrill, S. J. Falck and D. L. Harmon. (2011), 'Protein Supplementation of Ruminants Consuming Low-Quality Cool-or Warm-Season Forage: Differences in Intake and Digestibility', *Journal of animal science* Vol. 89, No. 11, pp. 3707-3717. doi:10.2527/jas.2011-3915

Burd, N. A., C. F. McKenna, A. F. Salvador, K. J. M. Paulussen and D. R. Moore. (2019), 'Dietary Protein Quantity, Quality, and Exercise Are Key to Healthy Living: A Muscle-Centric Perspective across the Lifespan', *Frontiers in Nutrition* Vol. 6, pp. 461773. https://doi.org/10.3389/fnut.2019.00083

Currier, T. A., D. W. Bohnert, S. J. Falck and S. J. Bartle. (2004), 'Daily and Alternate Day Supplementation of Urea or Biuret to Ruminants Consuming Low-Quality Forage: I. Effects on Cow Performance and the Efficiency of Nitrogen Use in Wethers', *Journal of Animal Science* Vol. 82, No. 5, pp. 1508-1517. doi: 10.2527/2004.8251508x.

Deng, K., T. Ma, Z. Wang, W. TanTai, H. Nie, Y. Guo, F. Wang and Y. Fan. (2018), 'Effects of Perilla Frutescens Seed Supplemented to Diet on Fatty Acid Composition and Lipogenic Gene Expression in Muscle and Liver of Hu Lambs', *Livestock Science* Vol. 211, pp. 21-29. https://doi.org/10.1016/j.livsci.2018.03.001

Deshmukh, S. S., B. H. Srikanth, G. U. Manju, D. Nandhini, R. Revannavar, J. K. Pavana, S. Pradeep and S. Sridhara. (2024), 'Nutritional Composition of Black Soldier Fly, Hermetia Illucens (L.) During Various Life Stages Reared on Vegetable and Fruit Waste', *Entomological News* Vol. 131, No. 2, pp. 121-128. https://doi.org/10.3157/021.131.0204

Duncan, D. B. (1955). Multiple ranges and multiple F-test. Biometrics, 11: 1- 42.

Fukuda, E. P., J. R. Cox, T. A. Wickersham and M. L. Drewery. (2022), 'Evaluation of Black Soldier Fly Larvae (Hermetia Illucens) as a Protein Supplement for Beef Steers Consuming Low-Quality Forage', *Translational Animal Science* Vol. 6, No. 1, pp. txac018. https://doi.org/10.1093/tas/txac018

Hsu, H. H. T. and N. C. Culley. (2006), 'Effects of Dietary Calcium on Atherosclerosis, Aortic Calcification, and Icterus in Rabbits Fed a Supplemental Cholesterol Diet', *Lipids in Health and Disease* Vol. 5, pp. 1-9. doi:10.1186/1476-511X-5-16.

Hussein, H. S. and R. M. Jordan. (1991), 'Fish Meal as a Protein Supplement in Ruminant Diets: A Review', *Journal of Animal Science* Vol. 69, No. 5, pp. 2147-2156. https://doi.org/10.2527/1991.6952147x

Jayanegara, A., B. Novandri, N. Yantina and M. Ridla. (2017a), 'Use of Black Soldier Fly Larvae (Hermetia Illucens) to Substitute Soybean Meal in Ruminant Diet: An in Vitro Rumen Fermentation Study', *Veterinary world* Vol. 10, No. 12, pp. 1439. DOI: 10.14202/vetworld.2017.1439-1446

Jayanegara, A., N. Yantina, B. Novandri, E. B. Laconi and M. Ridla. (2017b), 'Evaluation of Some Insects as Potential Feed Ingredients for Ruminants: Chemical Composition, in Vitro Rumen Fermentation and Methane Emissions', *Journal of the Indonesian Tropical Animal Agriculture* Vol. 42, No. 4. DOI: 10.14710/jitaa.42.4.247-254

Kahraman, O., N. Gülşen, F. İnal, M. S. Alataş, Z. S. İnanç, İ. Ahmed, D. Şişman and A. E. Küçük. (2023), 'Comparative Analysis of in Vitro Fermentation Parameters in Total Mixed Rations of Dairy Cows with Varied Levels of Defatted Black Soldier Fly Larvae (Hermetia Illucens) as a Substitute for Soybean Meal', *Fermentation* Vol. 9, No. 7, pp. 652. https://doi.org/10.3390/fermentation9070652

Kearl, L. C. (1982). Nutrient Requirements of Ruminants in Developing countries. International Feedstuff Institute, Utah Agricultural Experiment Station, Utah State University, Logan, Utah, USA.

Lu, S., N. Taethaisong, W. Meethip, J. Surakhunthod, B. Sinpru, T. Sroichak, P. Archa, S. Thongpea, S. Paengkoum and R. A. P. Purba. (2022), 'Nutritional Composition of Black Soldier Fly Larvae (Hermetia Illucens L.) and Its Potential Uses as Alternative Protein Sources in Animal Diets: A Review', *Insects* Vol. 13, No. 9, pp. 831. DOI: 10.3390/insects13090831

Marono, S., G. Piccolo, R. Loponte, C. Di Meo, Y. A. Attia, A. Nizza and F. Bovera. (2015), 'In Vitro Crude Protein Digestibility of Tenebrio Molitor and Hermetia Illucens Insect Meals and Its Correlation with Chemical Composition Traits', *Italian journal of animal science* Vol. 14, No. 3, pp. 3889. https://doi.org/10.4081/ijas.2015.3889

Odongo, E. E., W. K. Bbosa and P. K. Kahunde. (2024), 'Black Soldier Fly (Bsf): A Sustainable Solution for Protein, Waste Management, and a Circular Bio-Economy', *European Journal of Theoretical and Applied Sciences* Vol. 2, No. 3, pp. 822-834. https://doi.org/10.59324/ejtas.2024.2(3).64

Owens, F. N., S. Qi and D. A. Sapienza. (2014), 'Invited Review: Applied Protein Nutrition of Ruminants—Current Status and Future Directions', *The Professional Animal Scientist* Vol. 30, No. 2, pp. 150-179. https://doi.org/10.15232/S1080-7446(15)30102-9

Phesatcha, B., K. Phesatcha, B. Viennaxay, M. Matra, P. Totakul and M. Wanapat. (2022), 'Cricket Meal (Gryllus Bimaculatus) as a Protein Supplement on in Vitro Fermentation Characteristics and Methane Mitigation', *Insects* Vol. 13, No. 2, pp. 129. https://doi.org/10.3390/insects13020129

Pinho, R., E. M. Santos, J. S. de Oliveira, G. G. P. de Carvalho, J. P. Alves, A. J. d. S. Macêdo, G. A. Pereira, D. M. Pereira, A. F. Perazzo and A. d. M. Zanine. (2019), 'Relationship between Forage Neutral Detergent Fiber and Non-Fibrous Carbohydrates on Ruminal Fermentation Products and Neutral Detergent Fiber Digestibility in Goats', *Revista Colombiana de Ciencias Pecuarias* Vol. 32, No. 2, pp. 126-138. https://doi.org/10.17533/udea.rccp.v32n2a06

Polat, E. S. (2000), *The Role of Protein Supplementation in Manipulation of Body Composition of Lamb*, University of Melbourne, Department of Agriculture and Resource Management.

Rehman, K. u., C. Hollah, K. Wiesotzki, R. U. Rehman, A. U. Rehman, J. Zhang, L. Zheng, T. Nienaber, V. Heinz and K. Aganovic. (2023), 'Black Soldier Fly, Hermetia Illucens as a Potential Innovative and Environmentally Friendly Tool for Organic Waste Management: A Mini-Review', *Waste Management & Research* Vol. 41, No. 1, pp. 81-97. DOI: 10.1177/0734242X221105441

Runa, R. A., M. M. Islam, M. Hasan and M. A. Akter. (2022), 'Blood Biochemical Parameters of Murrah Buffalo (Bubalus Bubalis) Reared in the High Salinity Area of Bangladesh', *Journal of Advanced Veterinary and Animal Research* Vol. 9, No. 4, pp. 736. doi: 10.5455/javar.2022.i643

Saddick, E. I., U. A. Nayel and E. E. Eldahshan. (2024), 'Evaluation of Shea Butter Supplementation in Finishing Lambs' Diets', *Egyptian J. Nutrition and Feeds* Vol. 27, No. 2, pp. 161-173. doi:10.21608/ejnf.2024.377413

SAS (2002). Statistical Analysis Systems Institute Inc., Release 8.1, Cary, NC., USA.

Schneider, B. H. and P. W. Flatt (1975). The evaluation of Feeds through Digestibility Experiments the Univ. of Georgia Press Athens 30602.

Sung, H. G., Y. Kobayashi, J. Chang, A. Ha, I. H. Hwang and J. K. Ha. (2006), 'Low Ruminal Ph Reduces Dietary Fiber Digestion Via Reduced Microbial Attachment', *Asian-Australasian Journal of Animal Sciences* Vol. 20, No. 2, pp. 200-207. https://doi.org/10.5713/ajas.2007.200

Van Huis, A. (2013), 'Potential of Insects as Food and Feed in Assuring Food Security', *Annual review of entomology* Vol. 58, No. 1, pp. 563-583. https://doi.org/10.1146/annurev-ento-120811-153704

Van Keulen, J. and B. A. Young. (1977), 'Evaluation of Acid-Insoluble Ash as a Natural Marker in Ruminant Digestibility Studies', *Journal of animal science* Vol. 44, No. 2, pp. 282-287. https://doi.org/10.2527/jas1977.442282x

Varga, M. (2014), 'Textbook of Rabbit Medicine', *(No Title)*. ISBN 0 7506 4002 2

Wang, Y., L. Jin, Q. N. Wen, N. K. Kopparapu, J. Liu, X. L. Liu and Y. G. Zhang. (2016), 'Rumen Degradability and Small Intestinal Digestibility of the Amino Acids in Four Protein Supplements', *Asian-Australasian journal of animal sciences* Vol. 29, No. 2, pp. 241. doi: 10.5713/ajas.15.0342

Wyss, M. and R. Kaddurah-Daouk. (2000), 'Creatine and Creatinine Metabolism', *Physiological reviews* Vol. 80, No. 3, pp. 1107-1213. DOI: 10.1152/physrev.2000.80.3.1107

Xia, C., M. A. U. Rahman, H. Yang, T. Shao, Q. Qiu, H. Su and B. Cao. (2018), 'Effect of Increased Dietary Crude Protein Levels on Production Performance, Nitrogen Utilisation, Blood Metabolites and Ruminal Fermentation of Holstein Bulls', *Asian-Australasian journal of animal sciences* Vol. 31, No. 10, pp. 1643. doi: 10.3390/ani14030469

Yu GuoHui, Y. G., C. Y. Chen YanHong, Y. Z. Yu ZiNiu and C. P. Cheng Ping. (2009), 'Research Progress on the Larvae and Prepupae of Black Soldier Fly Hermetia Illucens Used as Animal Feedstuff'. ISSN (Print): 0452-8255

Zhao, X., S. Ali, M. F. Hassan, M. A. Bashir, X. Ni, C. Lv, H. Yang, B. Danzeng and G. Quan. (2023), 'Effects of Graded Levels of Dietary Protein Supplementation on Milk Yield, Body Weight Gain, Blood Biochemical Parameters, and Gut Microbiota in Lactating Ewes', Frontiers in Veterinary Science Vol. 10, pp. 1223450. https://doi.org/10.3389/fvets.2023.1223450

تقييم إضافة يرقات ذبابة الجندي الأسود منزوعة الدهن إلى علائق عجول الجاموس النامية

أحمد عبد الله مروان[1]، إيمان إبراهيم صديق[2] و أسامة أبوالعز نايل[2]

[1]قسم الإنتاج الحيواني، كلية الزراعة، جامعة عين شمس، مصر

[2]قسم الإنتاج الحيواني، كلية الزراعة، جامعة المنوفية، شبين الكوم، مصر

تهدف هذه الدراسة إلى تقييم إضافة يرقات ذبابة الجندي الأسود المجففة منزوعة الدهن في علائق عجول الجاموس النامية. تم تجهيز يرقات الذباب لتتم إضافتها على هيئة مسحوق مجفف. تم تقسيم 15 عجل جاموسي بعمر 5 أشهر، وبمتوسط وزن 122.44 ± 12.26 كجم، إلى ثلاث مجموعات تجريبية في تصميم القطاعات العشوائية، وتلقت ثلاث علائق تجريبية على النحو التالي: العليقة الأولى تمثل العليقة الأساسية بدون أي اضافة، في حين تمثل العليقة الثانية والثالثة نفس العليقة الأساسية الأولى مضاف إليها 5 و10 كجم/طن يرقات الذباب المجفف، على التوالي. تم اجراء تجربة النمو لحساب متوسط النمو اليومي (ADG) على فترات كل اسبوعين، وذلك خلال 90 يومًا. وأيضا تجربة هضم للتحقق من قابلية هضم العناصر الغذائية باستخدام طريقة الرماد غير القابل للذوبان في الأحماض (AIA). ومن ثم تسجيل كمية المادة الجافة المأكولة ومعدل تحويل المادة الجافة (كجم مادة جافة/كجم زيادة). أظهرت النتائج أن إضافة يرقات ذبابة الجندي الأسود بمعدل10 كجم/طن أدت إلى تحسين معاملات جميع هضم العناصر الغذائية، ومعدل النمو اليومي بشكل معنوي ($p \leq 0.05$) مقارنة بمجموعة إضافة 5 كجم / طن والمجموعة القياسية، أيضا فان إضافة يرقات ذبابة الجندي الأسود بمعدل 5 كجم/طن أدت إلى زيادة معدل النمو اليومي مع تحسن في معامل هضم مستخلص الأثير (EE) والمستخلص الخالي من النيتروجين (NFE) مقارنة بالمجموعة القياسية. لم تكن هناك آثار ضارة على وظائف الكبد أو الكلى بالنسبة لكلا المستويين من إضافة اليرقات، أكدت النتائج أن إضافة اليرقات أدت إلى زيادة تركيز الدهون الثلاثية في بلازما الدم، وعلى العكس كان لها القدرة على خفض تركيز الكوليسترول في بلازما الدم.

بشكل عام يمكن إضافة يرقات ذبابة الجندي الأسود المنزوعة الدهن إلى علائق عجول الجاموس النامية لقدرتها على تحسين معاملات الهضم ومعدل النمو اليومي دون أي تأثير سلبي على وظائف الكبد والكلي.