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The Immature Stages of *Sarcophaga dux* (Diptera; Sarcophagidae): A Proposed Nutritional Source for Poultry

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ABSTRACT

Background: Insects, particularly those belonging to the order Diptera, offer a promising alternative protein source for poultry with numerous advantages. Among these, *Sarcophaga dux* (Diptera: Sarcophagidae), a widely distributed global dipteran species, including Saudi Arabia, exhibits potential for waste management. This study was conducted to investigate the nutritional values of both larval and pupal stages of *S. dux* as well as the feed intake (FI) and the feed conversion ratio (FCR). *S. dux* flies (≈170 to 200 newly hatched larvae) were reared in standardized laboratory conditions until reaching the pupal stage.

Methods: The daily feed intake was calculated for each larvae replicate and FCR was measured on the last day of the 3rd larval instar. Larvae and pupae from each replicate were subject to the proximate analysis, amino acids and minerals. Results indicated a progressive increase in FI throughout the development period, except for the last day of the 3rd larval instar.

Result: The FCR demonstrated a higher value comparing to previous study. Furthermore, both larval and pupal stages exhibited high crude protein, lipid content and crude fibre with low dry matter and elevated ash content. The amino acid and mineral contents were also high, with low levels of heavy metals. Notably, the pupal stage displayed superior results in this study. These results may indicate that larvae and pupae of *S. dux* are promising sources of protein, minerals and potentially other essential nutrients which, in fact, suggest it as a nutritionally valuable food sources for poultry. Upon comparing the larval and pupal stages, it is evident that essential minerals are generally abundant in both stages except for manganese (Mn), which was found to be highest in pupae. However, it is noteworthy that the levels of heavy metals in both stages were low and exhibited no significant difference except for lead (Pb).

Key words: Amino acids, Minerals, Poultry, Proximate analysis, *Sarcophaga dux*.

INTRODUCTION

As the global population continues to increase and living standards improve, there is a growing demand for animal-derived protein (Van Broekhoven *et al.*, 2015; Khan, 2018). Currently, more than 820 million people suffer from undernourishment, two billion experience micronutrient deficiencies and an additional two billion are overweight or obese (Józefiak and Engberg, 2015). This places significant pressure on existing food systems and raises sustainability concerns. Atowa *et al.* (2021) project that the human population will reach 9.8 billion by 2050.

In poultry farming, feed efficiency is one of the most crucial factors in determining the cost of producing a kilogram of poultry meat. Feed accounts for 60-70% of the total production costs. Feed conversion ratio (FCR) and feed intake (FI), are both useful tools to evaluate feed efficiency and act as benchmark to estimate the profitability of a farm or industry. Overall, understanding the factors that influence feed efficiency is crucial for optimizing animal for example increase poultry production and reducing feed costs (Prakash *et al.,* 2020).

To address these challenges and meet the growing demands, there is a substantial need for new and sustainable, cost-effective food sources with high-quality protein and other nutritional values to ensure human food security (Józefiak *et al.,* 2016).

Different insect Orders can be used as food sources such as Diptera (black soldier fly and housefly), Coleoptera (mealworms), Lepidoptera (silkworm) and Orthoptera (grasshoppers, locust and crickets). However, insects belong to order Diptera, especially black soldier flies (BSFs) and houseflies, are considered the most promising candidates for food and feed production (Pretorius, 2011; Rumpold and Schlüter, 2013).

Sarcophagidae are a large fly family and are commonly known as flesh flies due to their food demand as they feed

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on live and dead tissues (Al-Ghamdi *et al.,* 2018). *Sarcophaga dux* has a high prevalence in different parts of the world: North Africa, Southern Europe and Australia and Asia including Saudi Arabia (Al-Ghamdi *et al.*, 2018; Kumar *et al.*, 2021). *S. dux* is a holometabolous viviparous insect, as females lay first larval instar on suitable organic matter (Kumar *et al.,* 2021). It is of medical, veterinary and forensic importance as well as its role in ecology. In the field of ecology. Flesh fly larvae are particularly well-suited for converting manure into non-polluted residue as their ability to break down complex organic molecules into simpler molecules, which helps in reduce its harmful environmental impact (Hasan and Leong, 2018).

To date, limited information exists regarding the nutritional value of various life stages of Sarcophagidae species. The current project seeks to assess the nutritional content of both the larval and pupal stages of *S. dux* found in Saudi Arabia. Additionally, the project aims to estimate both the feed intake and the feed conversion ratio for these stages.

MATERIALS AND METHODS

Insects' collection and identification

Adult flies of (*Sarcophaga dux*) were collected from the education farm of King Saud University, Riyadh, Saudi Arabia (24° 44′ 13.42″ N, 46° 37′ 12.82″ E) according to Alotaibi *et al.* (2020). Flies were identified morphologically according to the keys of Sukontason *et al.* (2010) and Jeevan *et al.* (2024). These insects are of targeted species which were reared at insectary of King Abdulaziz City for Science and Technology according to Zhang *et al.* (2020).

Feed conversion ratio and intake

Feed intake (FI) in grams was calculated by recording the consumed amount of feed (chicken liver) each day. Initially, on the first day, 20 g of new chicken liver were provided to each of the three replicates, each group having of 10 newly emerged larvae. After 24 hours, the chicken liver was weighed and FI was determined for the first day by assessing the difference in liver weight before and after consumption by the larvae. Subsequently, new 20 g of fresh chicken liver was replaced and the FI was measured each day.

Similarly, the feed conversion ratio (FCR) was calculated by the total feed consumed during larval age/ weight gain during larval age (Bawa *et al.,* 2020).

Dry matter determination

The dry matter (DM) of larvae and pupae were estimated according to Pretorius (2011). Each group having weight of 2 g was placed in petri dishes and dried in oven (Carbolite oven, CARBOLITE GERO, UK) for 24 hours for larval and 12 hours for pupal stage at 65°C. Then, the dry sample was weighed and the DM content was measured using following formula:

$$\text{Moisture (\%)} = \frac{W1\text{-}W2}{S} \times 100$$

Where,

W1 = Weight of the petri dish with wet sample (before drying) (g).

W2 = Weight of the petri dish with dried sample (after drying) (g).

S = Weight of the wet sample (g).

$$\text{Dry matter (\%)} = 100 - \text{Moisture}$$

Ash determination

The milled dried samples (larvae and pupae) of each replicate were combusted in a combustion oven (Vulcan 3-1750, Cole-parmer, US) according to Pretorius, (2011) at 550°C overnight. The combusted samples were weighed and the ash was determined according to the following equation:

$$\text{Ash (\%)} = \frac{(A\text{-}B)}{S} \times 100$$

$$\text{Organic matter (\%)} = 100 - \text{Ash}$$

Where,

A = Weight of crucible and ash (g).

B = Weight of empty and dry crucible (g).

Evaluation of crude protein

Briefly, this method comprises three essential steps: digestion, distillation and titration. Initially, the samples underwent digestion at 420°C by adding 14 ml of concentrated sulfuric acid and Kjeltabs Cu/3,5 (Foss, Denmark) per 0.5 g of each sample. The digestion process took place in a Tecator 2020 digester (Gemini sustainable laboratory equipment, Dutch). Subsequently, distillation and titration were carried out using the Kjeltec sampler 8420 (Foss, Denmark) and the nitrogen content was directly measured. The protein content was calculated using the following equation:

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Evaluation of crude fat

It was dried in drying oven (Carbolite oven, CARBOLITE GERO, UK) for 15 min at 100°C. The fat content was calculated using the following equation:

$$\text{Crude fat (\%)} = \frac{(A\text{-}B)}{S} \times 100$$

Where,

A = Weight of beaker after extraction (g).

B = Weight of empty beaker (g).

Evaluation of crude fibre

The fibre content was determining according Pretorius, (2011). In 500 ml beaker, 1g of dried milled larvae and pupae of each replicate was placed with 50 ml of H_2SO_4 (5%) and completed to 200 ml with distil water. Then, placed in Tecator Soxtec System (Crude Fiber Apparatus, Labconco, US) for 30 min. Thereafter, the samples washed with distilled water and placed in same beaker with 50 ml of NaOH (5%) and 200 ml with distil water was added and set at Tecator Soxtec

System for 30 min. Then, samples were washed with distilled water and placed in crucible and dry them in drying oven (Carbolite oven, CARBOLITE GERO, UK) at 100°C over-nights. In the next day, the samples combusted in a combustion oven (Vulcan 3-1750, Cole-parmer, US) at 500°C for 5 hr. the crude fibre was determined according the following equation:

$$\text{The crude fiber (\%)} = \frac{(A-B)}{S} \times 100$$

Where:
A = Weight of crucible after dried at 100°C (g).
B = Weight of crucible after dried at 500°C (g).

Evaluation of amino acid

The samples (dried milled larvae and pupae of each replicate) were sent to the Arabian Agricultural Services Company (ARASCO) IDAC Merieux (Riyadh, Saudi Arabia) (https://www.arasco.com/idac-merieux/) and analysed according to the method described by Association of Official Analytical Chemists International (AOAC), Official Method 2007.01 (Lehotay, 2023).

Evaluation of minerals

Mineral were analysed according to Pretorius, (2011). A lower FCR indicates better efficiency; for example, if an animal needs less feed to gain a kilogram of body weight, its FCR is lower and it's considered more efficient. However, High FCR itself doesn't imply nutritional value; it mainly reflects the efficiency of feed conversion. So, while a high FCR indicates that more feed is needed to achieve growth or weight gain, it doesn't directly reflect the nutritional value of the feed or the final food product. Nutritional value is about the quality and quantity of nutrients provided, rather than the efficiency of converting feed into weight (Bawa *et al.,* 2020).

Statistical analysis

Data from different 3 replicates (n = 3) of each stage (larval and pupal stage) were used for statistical analysis using SPSS software (version 22, IBM SPSS Statistics, 2013). Prior to any further analysis, data were first tested for normality using Anderson Darling Normality test. Upon being normally distributed data, a t-test was used for comparing differences between means in each experiment (Morrison, 2002). Significant differences were considered at $P \leq 0.05$.

Results and Discussion

The developmental period of *Sarcophaga dux*

The findings of the current study reveal that *S. dux* exhibits a short life cycle. Females of *S. dux*, reared under conditions of 28±5°C, 50±10% relative humidity and a 12:12-hour photoperiod, laid the first larval instar after 4 hours on a suitable substrate. In this environment the larval age was of 86 hours, while the pupal age required 232.67 hours for its completion. The entire duration from larval deposition to adult emergence was approximately 318.67 hours (Fig 1).

The larval body sizes

Data showed variations in body sizes among different larval instars, including differences in weight, width and length (Table 1). The results revealed a significant distinction between the 1st and the 3rd larval instars in terms of weight (0.75±0.15 and 148.13±1.78 mg, respectively), width (0.49±0.02 and 3.83±0.15 mm, respectively) and length (3.40±0.14 and 17.22±0.18 mm, respectively).

Table 1: The average mean of body measurements (length, width and weight) of first and third larval instars of *Sarcophaga dux*.

Body measurements of larval instars (Mean ± SE)			
Larval instar	Weight Mean (mg) ± S.E	Width Mean (mm) ± S.E	Length Mean (mm) ± S.E
1st	0.75^{a}±0.15	0.49^{a}±0.02	3.40^{a}±0.14
3rd (pre-pupae)	148.13^{b}±1.78	3.83^{b}±0.15	17.22^{b}±0.18

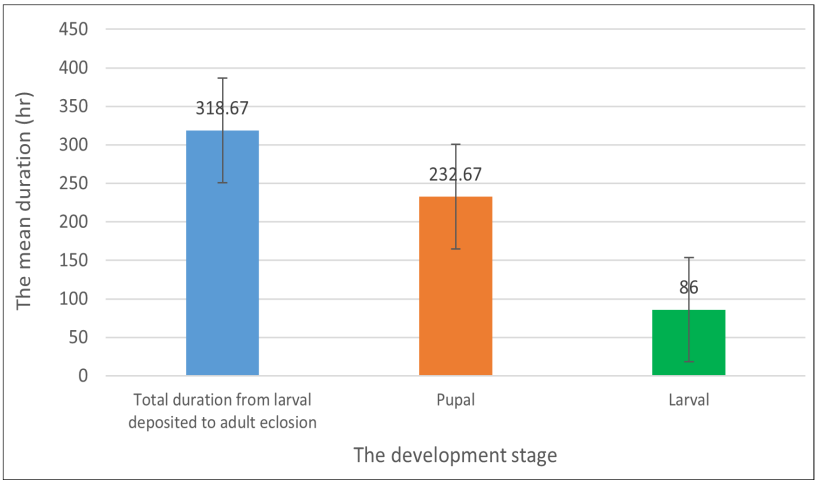

Fig 1: Durations in hours (the mean) of *Sarcophaga dux* life cycle.

Feed intake and the feed conversion ratio of larval stages

The feed intake ratios of *S. dux* larvae, as presented in Table 2, were (8.21±0.26, 10.49±0.07, 10.74±0.54 and 8.41±0.79 g) for the 1st larval instar in the 1st day, 2nd larval instar of the 2nd day, 3rd larval instar of the 3rd and 4th day, respectively. Significant differences were observed among different days, but surprisingly no significant variations were noted between the 1st and 4th day, as well as between the 2nd and 3rd day. Generally, the results suggest an increase in feed intake with growing larvae, except on the last day (4th day). Results also showed that the feed conversion ratio of *S. dux* larvae was 12.67.

Proximate analysis of larval and pupal stage

A significant variation was observed in the crude protein content between the larval and pupal stages. Pupae exhibited higher crude protein contents than larvae, with values of (55.50%±0.10 and 65.43%±0.53 respectively). The fat content showed higher value in larval stage with no significant difference. While, the crude fibre was higher in pupal stage with significant variation in studies stages (Table 3).

Amino acids profile of larval and pupal stage

As depicted in Table 4, both examined stages (larva and pupa) exhibited variations in amino acid composition. In the larval stage, the highest amounts of essential amino acids were tyrosine and lysine (5.51%±0.11 and 5.39%±0.67, respectively), while the non-essential amino acids included glutamic acid, glycine and arginine (6.49%±0.08, 4.57%±0.52, 5.35%±0.16, respectively). In the pupal stage, the highest essential amino acids were leucine and lysine (4.16%±0.18 and 5.27%±0.44, respectively) and the non-essential amino acids included glutamic acid, glycine and arginine (6.25%±0.15, 6.26%±0.25, 6.15%±0.04, respectively).

Upon comparing the larval and pupal stages, both stages exhibited the same number of amino acids; however, there were significant differences ($p<0.05$) in the quantity of glycine, arginine, tyrosine, alanine and isoleucine. Overall, the amino acid composition of the pupal stage was superior to that of the larval stage (58.96%±0.64).

Mineral and Heavy metals contents of larval and pupal stage

As presented in Table 5 in the larval stage, most essential minerals, apart from zinc, exhibited high values, including Cu, Mg, Mn and Na (11.87 ppm±0.64, 12.78 ppm±1.20, 20.92 ppm±0.63 and 11.03 ppm±3.92, respectively). Notably, potassium (K) was the highest mineral overall in the larval stage (90.34 ppm±6.85). In contrast to essential minerals, heavy metals were present in relatively low amounts (0.03 ppm ± 0.01, 0.02 ppm±0.00, 0.40 ppm±0.08, 0.21 ppm ±0.04) for arsenic, cadmium, chromium and lead, respectively. Like the larval stage, the pupal stage exhibited high values for most essential minerals (Cu, Mg, Mn and Na) (13.18 ppm ± 0.26, 12.82 ppm ± 0.15, 25.14 ppm ± 0.53 and 6.66 ppm ± 0.54, respectively). Potassium (K) remained the highest essential mineral (71.70 ppm ± 2.63), while zinc showed the lowest concentration. Heavy metals were also present in low quantities in the pupal stage

Table 2: The average mean of feed intake estimated in each day at different larval instars of *Sarcophaga dux*.

	The feed intake (g) (Mean ± S.E)
1st day	8.21^{a}±0.26
2nd day	10.49^{b}±0.07
3rd day	10.74^{b}±0.54
4th day	8.41^{a}±0.79

SE: Standard errors.

Table 3: The proximate analysis of larval and pupal stage of *Sarcophaga dux*.

Parameters	Larval stage Mean (%) ± S.E	Pupal stage Mean (%) ± S.E
Moisture	71.23^{b}±0. 23	66.45^{a}±0.27
Dry matter	28.77^{a}±0.23	33.55^{b}±0.27
Ash	3.94±0.07	4.23±0.71
Organic matter	96.06±0.07	95.77±0.71
Crude protein	55.50^{a}±0.10	65.43^{b}±0.53
Crude fat	19.23±4.43	13.73±1.43
Crude fiber	8.48^{a}±0.24	20.30^{b}±1.21

SE: Standard errors.

Table 4: Amino acids composition (%) of *Sarcophaga dux* in larval and pupal stage.

The amino acids profile	Mean ± S.E(%)	
	Larvae	Pupae
Essential amino acids		
Histidine	3.47±0.28	3.77±0.12
Tyrosine	5.51^{b}±0.11	3.99^{a}±0.08
Cystine	0.25±0.01	0.26±0.02
Valine	2.32±0.04	2.46±0.08
Methionine	2.53±0.10	2.79±0.01
Phenylalanine	3.18±0.10	3.08 ±0.13
Isoleucine	1.68^{a}±0.05	1.98^{b}±0.08
Leucine	3.86±0.08	4.16±0.18
Lysine	5.39±0.67	5.27±0.44
Threonine	2.05±0.03	2.06±0.07
Non-essential amino acids		
Aspartic acid	3.75±0.05	3.68±0.12
Glutamic acid	6.49±0.08	6.25±0.15
Serine	2.67±0.07	2.85±0.20
Glycine	4.57^{a}±0.52	6.26^{b}±0.25
Arginine	5.35^{a}±0.16	6.15^{b}±0.04
Alanine	2.68^{a}±0.03	2.82^{b}±0.04
Proline	1.23±0.11	1.12 ±0.05
Total amino acid	56.97^{a} ±0.13	58.96^{b} ±0.64

SE: Standard errors.

Table 5: The mineral and heavy metals composition of larval and pupal stage of *Sarcophaga dux*.

	Mean (PPM) ± S.E	
	Larval stage	Pupal stage
The essential minerals		
Cu	1.87±0.64	13.18±0.26
K	90.34±6.85	71.70±2.63
Mg	12.78±1.20	12.82±0.15
Mn	20.92^{a}±0.63	25.14^{b}±0.53
Na	11.03±3.92	6.66±0.54
Zn	0.44±0.06	0.44±0.02
The heavy metals		
As	0.03±0.01	0.02±0.00
Cd	0.02±0.00	0.02±0.00
Cr	0.40±0.08	0.35±0.04
Pb	0.21^{b}±0.04	0.10^{a}±0.01

(0.02 ppm ±0.00, 0.02 ppm ± 0.00, 0.35 ppm ±0.04 and 0.10 ppm ± 0.01) for arsenic, cadmium, chromium and lead, respectively.

The ash in the current study was higher in pupal stage (4.23%) while, the organic matter was greater in larval stage (96.06%), this indicates a greater concentration of minerals in larval stage compared to organic matter in the pupae (Bednářová *et al*., 2013). Bednářová *et al*., (2013) documented the ash value in *Gryllus assimillis* nymphs (4.26%) which are in consistence to *S. dux* pupae (4.23%). Pretorius. (2011) who study *Musca domestica* as feed for poultry, recorded ash as (10.68%) in larvae and (7.73%) in pupal stage, both stages show higher ash content than the present study (3.937 and 4.233% respectively).

The analysis of crude protein content in this study reveals that both stages of *S. dux,* the larval stage (55.50%) and the pupal stage (65.43%), exhibit higher protein content compared to soymeal (51.8%) (Makkar *et al*., 2014; Elahi *et al*., 2022: Gangil *et al.,* 2021). Specifically, the crude protein content of *S. dux* larvae (55.50%) surpasses that observed in the larvae of *Hermetia illucens* (42.1%), *Musca domestica* (50.4%) and *Tenebrio molitor* (52.8%) (Makkar *et al*., 2014, Khan 2018 and El-Hentati *et al*., 2023) notes that the crude protein content of *Hermetia illucens* ranges between 41.1% and 43.6%, while Chu *et al*. (2020) demonstrated a lower crude protein value of 34.97% for *Hermetia illucens* larvae. Hall *et al*. (2018) reported a crude protein value of 53.5% in *Musca domestica* larvae. Study of Smets *et al.* (2020) in *Hermetia illucens* pupae showed higher value of lipid than present study (39.85%).

Additionally, comparisons were made with a previous study on other insect species. Notably, all the studied heavy metals in *S. dux* were found to be below the maximum allowable values. In a study by Purschke *et al.* (2017) and Panda *et al.* (2005)on *Hermetia illucens* larvae, the values of arsenic, cadmium, chromium and lead were reported as 2.8 ppm, 13.7 ppm, 3.4 ppm and 35.6 ppm, respectively. These values were higher than those obtained in the larval stage of *S. dux* (0.03 ppm, 0.02 ppm, 0.40 ppm and 0.21 ppm, respectively) and the pupal stage (0.02 ppm, 0.02 ppm, 0.35 ppm and 0.10 ppm, respectively). This indicates that *S. dux* poses no significant safety concerns for consumption, whether as feed material, complete feed, or as a food source.

Conclusion

Conclusion the higher nutritional value including amino acid content and a good value of proximate analysis such as the high crude protein, low dry matter, high fat content, crude fibre and high ash content, with appropriate value of mineral and a low heavy metal of *S. dux* makes it a more valuable feed ingredient for poultry. Overall, the study found that flies are a good source of protein and amino acids, this makes them a potential feed source and more research is needed to confirm the nutritional value of flies and to ensure that they are safe to eat.

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Conflicts of Interest

All authors declare that they have no conflict of interests in this manuscript.

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