EFFECTS OF DIFFERENT DIETS ON LIFE PARAMETERS AND NUTRITIONAL PROFILE OF MEALWORMS (*TENEBRIO MOLITOR* L) UNDER LABORATORY CONDITIONS

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ABSTRACT

This study was planned to investigate the effect of different diets on biological parameters and nutritional profile of *Tenebrio molitor* under lab condition (26 ± 1 °C, 65 ± 5% RH and 14L :10D Photoperiod) Thirteen diets were developed from four different food sources, using two crop waste (i.e. (Corn straw, rice straw), wheat bran and yeast. These four main ingredients were mixed in different ratios (w/w). A total of 780 (7 days old) mealworm larvae obtained from stock culture and reared on thirteen different experimental diets according to a completely randomized design with three replicates (n=20). Results revealed that biological parameters and nutritional profile of *T. molitor* were significantly affected by the tested diets. However, standard diet (wheat bran 95%+yeast 5%) followed and Diet -5 (Rice straw 40% + wheat bran 55% + Yeast 5%) appeared to be the most effective diet have maximum larval weight gain (147.14±2.29mg and125.46±2.62mg), bigger larvae (27.563±0.81mm and24.68±0.74mm) and quick larval development of 83.01±1.62days and 89.91±1.34days respectively. Similar trend was observed for the pupal and adult development. The standard diets resulted in heavier pupae and adults as compared to all other tested diets. In general, the adult reproduction was clearly favored by most of diets with higher percentage of wheat bran which was in contrast to the diets composed of only the crop residues (D-11 and D-12) on which lowest number of eggs was laid by the females. Larval survival rate ranged from 87.95- 95.81% on the selected diets. A nutritional profile of *T. molitor* larvae was evaluated after 60 days of feeding trial. Post-feeding nutritional proximate analysis showed significant differences in nutritional contents. *T. molitor* demonstrated highest moisture (11.93±0.10%) in Diet-8. On the other hand, *T. molitor* showed highest crude protein (57.21±0.91%) when offered Diet-13followed by Diet 5 with crude protein content of 48.33±0.60 % and higher level of fiber (13.13±0.29%) with Diet-12. Fat content was also noticed higher in the Diet-12 (34.02±0.99%) whereas ash (7.92±0.17%) and NEF (13.08±0.13%) was highest in Diet-8 and Diet-4respectively. It was concluded that standard diet (Diet-13) and Diet-5 having high amount of protein and larvae fed upon these diets resulted in better weight, length, duration and survival rate along with rich amount of protein contents. Hence for mass rearing and quality production of mealworms the above diets can be utilized. It was concluded from these results that Diet-13 and Diet-5 have remarkable effect on biological parameters and nutritional profile particularity the protein content, thus recommended for mass production of *T. molitor*.

Keywords: Mealworm; biological parameters; Wheat bran; rice straw; crude protein; Nitrogen Free Extract (NFE)

INTRODUCTION

The current population explosion will lead for higher food demand in the coming years. Protein rich food demand will also be increased. The expected population will be nine billion by the end of 2050 (Markkar *et al.*, 2014). To meet such demands of the population the world is shifting to other sources of food containing rich amount of protein. Insect rearing as a novel protein source for animal feed and human food has been gaining popularity among the people and already existed production projects at an industrial level (van Huis *et al.*, 2013). Insects are promising feed for animal because they have short life cycles, contain high quantities of proteins and can easily breed and handle depending on the substrates used for their production (Ramos-Elorduy *et al*., 2002).

Over 1,700 insect species are consumed as a food globally (Johnson, 2010; FAO, 2010). Some of the important species are *Tenebrio molitor* (yellow mealworm), *Alphitobius diaperinus* (lesser mealworm), *Zophobas morio* (giant mealworm), *Galleria mellonella* (greater wax moth), *Acheta domesticus* (house cricket), *Bombyx mori* (silkworm) and *Locusta migratora* (African migratory locust) that have the greatest potential to be used as food and feed (EFSA, 2015). Among these insects, *T. molitor* is gaining great attention as a protein source for food purposes throughout the world (Van Huis *et al.*, 2013). These insects contain higher protein contents than plant protein including eighteen kinds of essential amino acids, vitamins and minerals (Jin *et al.*, 2016). They are usually served live, sold in dried, canned or processed powdered form (Veldkamp *et al.*, 2012).

Mealworms contain higher amount of crude protein (25-60%) and fats (15-40%) (Khan *et al.,* 2017; DeFoliart *et al.,* 2009). The *Tenebrio molitor* can also be used as alternative source of nutrition for broiler chickens. The major factors that affect the high cost of poultry feeds are the scarcity of feed ingredients soya-bean, maize and fishmeal (Adeniji

2007). Due to increase in prices of conventional protein sources such as soybean, fish and meat, insects have been focused as an alternative source of protein because of their high nutritive profile and essential amino acids (Ng *et al*., 2001; Karimi, 2006; Ravzanaadii *et al.*, 2012).

T. molitor larvae are usually fed with wheat bran, corn, oat, wheat flour and supplemented with other protein sources, such as poultry feed and powdered milk. Fruits or vegetables can also be included in the diet as a source of moisture and grain residues (Nguyen *et al.* 2015). However, the larvae can also feed on organic waste, with the potential of recycling lost nutrients by incorporating residual amino acids, fatty acids from manure, and organic waste into their biomass (Oonincx *et al.* 2015).

Mainly wheat bran, oat, corn wheat flour with addition to protein supplement are fed to the larvae of *T. molitor.* For moisture source vegetables and fruits can be added in the diets (Nguyen *et al.* 2015). Other organic waste can be given as a feed to *T. molitor* larva which they converted into their biomass (Oonincx *et al.* 2015).

T. molitor can utilized the agricultural by-products such as brewer corn straw, brewer's spent grain, rice straw, spent mushroom substrate, tangerine shell and wheat straw that have usually been trashed. With these benefits, many researchers are focusing on improving mealworm industry (Kim *et al.*, 2015; Li *et al.*, 2012). Fortunately, *T. molitor* can efficiently degrade the organic matters thus transforming the low quality organic wastes into the high quality protein products into the larval biomass (Van Huis *et al*., 2013; Van Broekhoven *et al*., 2015; Tan *et al*., 2018).

To rear the *T. molitor* on different low cost agriculture by products as alternative feeding substrate, the current study was planned to utilize the crop residues including the rice straw and corn straw alone or in combination with the wheat bran for mass production of mealworms and to investigate their effects on the growth performance and nutritional composition.

MATERIAL AND METHODS

Experiment on the effects of different diets on life parameters and nutritional profile of mealworms (*Tenebrio molitor*) was carried out under lab condition at the Department of Entomology, The University of Agriculture Peshawar-Pakistan.

Mealworm Culture raising:

Prior to experiment, stock culture of mealworm was maintained in an incubator 26 ± 1 °C, 65 ± 5% RH and 14L:10D(Photoperiod). Newly hatched (7 days old) *T. molitor* larvae were obtained from the Insect Rearing Laboratory of the Department of Entomology, The

University of Agriculture Peshawar. The larvae were kept in plastic rearing trays (30x20x25 cm) and provided with wheat bran as feeding substrate and potato, placed on top of each container to serve as a moisture source. Old feed was replaced with fresh feed once a week till pupation. Upon pupation, the pupae were collected and shifted in a rearing tray (size). Newly emerged adults were provided with wheat bran and potato. Same procedure was continued for maintenance of *T. molitor* culture

Diets Preparation

Diets used in the experiment were developed using four distinct feed sources, Corn straw (CS), rice straw (RS), Wheat bran (WB) and Yeast (Y). Corn straw and rice straw were acquired from farmer's field while wheat bran and yeast were purchased from a near by market. Prior to testing, CS and RS were cut into small pieces, washed with tape water and allowed to evaporate tape water under room temperature then oven dried at 80^{0} C for 48 hours. After drying, CS and RS were ground with the plant grinder and sieved through a 100 mesh sieve to make the ingredient more appealing for *T. molitor* larvae. Thirteen different diets (Table 1) were made by mixing powdered CS, RS, wheat bran and yeast in different ratio (weight/weight). Diets were kept in clean polyethene bags, tagged with diet name and kept at 4^{0}C in refrigerator for experiment.

Table 1 Diets used in the experiment

Diet	Composition	
D1	RS 20%+ CS 20%+ WB55%+ Y5%	(W/W)
D2	RS 40%+ CS 40%+ WB15%+ Y5%	(W/W)
D3	RS 40%+ CS 50%+ WB5%+ Y5%	(W/W)
D4	RS 50%+ CS 40%+ WB5%+Y5%	(W/W)
D5	RS 40%+ WB55%+Y5%	(W/W)
D6	CS 40%+ WB55%+Y5%	(W/W)
D7	RS 60%+ WB35%+Y5%	(W/W)
D8	CS 60%+ WB35%+Y5%	(W/W)
D9	RS 80%+ WB15%+Y5%	(W/W)
D10	CS 80%+ WB15%+Y5%	(W/W)
D11	RS 95%+Y5%	(W/W)

D12	CS 95%+Y5%	(W/W)
D13	**Standard diet:** WB 95%+Y 5%	(W/W)
Diets (%w/w) D_1: Rice Straw20%+ Corn Straw20%+ Wheat Bran55%+ Yeast5%, **D_2**: Rice Straw40%+ Corn Straw40% + Wheat Bran15%+ Yeast5%,**D_3**: Rice Straw40% + Corn Straw50% + Wheat Bran5%+ Yeast5%,**D_4**: Rice Straw50%+ Corn Straw40%+ Wheat Bran5%+Yeast5%,**D_5**: Rice Straw 40%+ Wheat Bran55%+Yeast 5%, **D_6**: Corn Straw40%+ Wheat Bran55%+Yeast5% **D_7**: Rice Straw60%+ Wheat Bran35%+Yeast5% **D_8**: Corn Straw60%+ Wheat Bran35%+Yeast5% **D_9**: Rice Straw80%+ Wheat Bran 15%+Yeast5%, **D_{10}**: Corn Straw80%+ Wheat Bran15%+Yeast5%, **D_{11}**: Rice Straw95%+Yeast5%, **D_{12}**: Corn Straw95%+Yeast5%, **D_{13}**: Wheat Bran 95%+Yeast 5%		

Feeding tail and Experimental Design:

Experiment was designed to evaluate the effect of different diets (Table 1) on life parameters of *T. molitor*. For this purpose, twenty(6 -7 days old) larvae were obtained from the stock culture and placed in plastic rearing tray(24 x 16.1 x 6 cm), were provided with 50 g of each diet along with fresh potato as moisture source. Rearing trays were tagged with diet name and covered with perforated plastic cover to prevent their escape. All rearing trays were kept in environmental chamber (Binder Environmental Chamber, Model No. 78532) at 26±1°C, 65% RH, and a photoperiod of 14:10 (L:D). The experiment was arranged in Completely Randomized Design (CRD) with three replications. Every 7 days, mealworm frass and shed skins were cleaned out with 80-mesh sieve and diets were refreshed respective diet in each rearing tray.

Data collection

Data on final weight, body length, duration and survival rate of larva, pupa and adult was recorded by randomly selecting five individuals from each diet. For female oviposition, a pair of newly emerged adults were collected from each diet and placed in a separate plastic tray lined with a white paper to facilitate the egg laying. The mated female started laying her eggs in diets on sheet provided at the bottom. The sheet with eggs (adhered) were checked and replaced daily with a new sheet in order to record the daily fecundity of female in each diet. The eggs were counted daily and then placed in a controlled environmental chamber for hatching. Average number of eggs laid by a single female during the oviosition period was also determined. The studied parameters were calculated by using the following formulae.

1. Weight gain (larva/pupa/adult) = Final weight (larva/pupa/adult)-Initial weight (larva/pupa/adult)
2. Length gain (larva/pupa/adult)= Final length (larva/pupa/adult)–Initial length(larva/pupa/adult)
3. Larval duration (days)= Date of last pupa formed - Date of first larva hatched
4. Pupal duration (days) = Date of last adult emerged- date of first pupa appeared
5. Adult longevity (days) = Date of adult death - date of emergence

6. Survival rate %age = $\frac{\text{Initial number of larvae} - \text{number of dead larvae}}{\text{Initial number of larvae}} \times 100$

7. Eggs/female/day = $\frac{\text{Number of eggs laid}}{\text{Number of days}}$

Nutritional profile of diets and *T. molitor* larvae:

Nutritional profile of the tested diets and *T. molitor* was carried out Chemistry Lab at Veterinary Research Institute (VRI), Peshawar. For nutritional analysis of *T. molitor*, mass culture of *T. molitor* was maintained on the diets mentioned in (Table 1). Same methodology was followed as mentioned in Feed testing trail. Later instar larvae were collected from the respective diets and boiled (100 °C) for 3 minutes, and then oven dried at 60 °C for 24 hours. After drying, mealworm larvae were ground into fine particles using an electric blender prior to the nutritional analysis. 10 gm sample of each diet and the respective meal of larvae were subjected for nutritional analysis. Three replicates were performed for each diet in the experiment. Moisture, crude protein, crude fats, ash, fiber content and NFE contents were determined according to (AOAC, 2000).

Statistical Analysis:

Data recorded on different parameters were submitted to ANOVA, means were separated by using the LSD test at 5% of level of significance (Steel and Torrie, 1980) using statistical package "Statistix 8.1".

RESULTS:

Larval parameters of *T. molitor*

Results presented in Table 2 showed that all larval parameters were significantly affected ($P<0.05$) by the tested diets. However, maximum weight gain (147.14±2.29 mg) was recorded in Diet13 (Wheat Bran 95% + Yeast 5%) followed by Diet 5 (Rice Straw 40%+ Wheat Bran 55% + Yeast 5%) and Diet1 (Rice straw 20%+ Corn straw 20%+ wheat bran 55%+ yeast 5%) with weight gain of 125.24±2.08 mg and 119.31+2.34mg respectively. while minimum weight gain (98.27±1.02 mg) was recorded in Diet 12 (Corn straw 95%+ Yeast 5%).

Larva attained maximum length in case of D-13 (27.56 ±0.81 mm) while the minimum larval weight was recorded in D-12 (16.11±0.89 mm) as shown in Table 2. Results further revealed that diets with higher percentage of wheat bran D-1, D-5, D-6 and.D-7.had produced bigger larvae (23.19±0.78 mm), (24.68±0.74 mm), (22.35±0.93 mm) and (22.67±0.61 mm) respectively as compare to other diets.

Significant variation in larval duration of *T. molitor* was observed when offered different larval diets (F=219, P=0.000) Table 2. Larval duration of *T. molitor* was significantly shorter (83.01±1.62 days) in D-13while the duration was significantly longer in D-10 (109.25±2.21 days). In general, diet with higher wheat bran shortened the developmental time for the mealworms larvae or vice versa. Longer larval developmental time was recorded in corn straw supplemented diets as compared to the diets having rice straw as a feeding substrate.

Percent survival rate of the *T. molitor* larvae on different diets under lab condition was also recorded Table 2. Results showed that survival rate of larvae was greatly affected by the diets composition. Significant variation (P=0.000) was recorded in survival rate of larvae. Maximum percent survival rate of the larvae was recorded in D-1 (95.79%), D-8 (95.33%) and D-13 (95.81%).A significantly minimum survival rate of larvae (88.37%) and (89.35%) was recorded in D-10 and D-12 respectively. Both these treatments were at par. The remaining diets showed an average survival rate of larvae. The survival rate of *T. molitor* larvae was above 90% in all diets except D-4, D-10and D-12where larval survival rate was 87.97%, 88.3% and 89.35% respectively.

Table 2. Larval parameters of *T. molitor* fed on different diets under laboratory conditions

Diet	Weight gain (mg) ± SD	Length (mm) ± SD	Duration (days) ± SD	Survival rate (%)± SD
D1	119.31±2.34 c	23.19±0.78 c	92.75± 3.34 g	95.79±1.61 a
D2	113.15±2.33ef	21.81±1.40 d	93.05±2.49 g	94.33 ± 2.06c
D3	110.21±0.89g	20.76±0.34 e	99.47±2.57 d	90.23 ±1.95fg
D4	101.37±1.65 h	18.21±0.63 h	101.47±1.07 c	87.95± 2.46h
D5	125.46±2.62 b	24.68±0.74 b	89.91±1.34 h	91.74 ± 1.97e
D6	115.24±2.08 de	22.35±0.93 cd	102.55±1.32 c	90.38 ± 1.49f
D7	117.28±1.86cd	22.67±0.61 c	97.88±1.74 e	94.67± 1.28bc
D8	111.53±2.65fg	20.23±0.87 e	104.72±1.61 b	95.33 ±2.31ab
D9	113.42±2.08ef	19.29±0.52 fg	95.73±1.39 f	92.90 ±1.90 d
D10	110.71±1.44 g	20.13±0.24 ef	109.25±2.21 a	88.37 ±2.44 h
D11	103.26±2.77 h	18.53±0.65 gh	101.53±2.93 c	91.67 ± 1.52e
D12	98.273±1.02i	16.11±0.89 i	105.33±1.13 b	89.35 ±2.60 g
D13	147.14±2.29 a	27.563±0.81 a	83.01±1.62 i	95.81 ± 1.19a

LSD(0.05)	2.4209	0.8470	1.3522	0.9571

Larval parameters of *T. moilitor* reared on D_1: RS 20%+ CS 20%+ WB55%+ Y5%, **D_2**: RS 40%+ CS 40%+ WB15%+ Y5%,**D_3**: RS 40%+CS 50%+ WB5%+ Y5%,**D_4**: RS 50%+ CS 40%+ WB5%+Y5%,**D_5**: RS 40%+ WB55%+Y5%, **D_6**: CS 40%+ WB55%+Y5% **D_7**: RS 60%+ WB35%+Y5% **D_8**: CS 60%+ WB35%+Y5% **D_9**: RS 80%+ WB15%+Y5%, **D_{10}**: CS 80%+ WB15%+Y5%, **D_{11}**: RS 95%+Y5%, **D_{12}**: CS 95%+Y5%, **D_{13}**: WB 95%+Y 5%
Means in column followed by different letter are significant at 0.05% level of significance followed by LSD test.

Pupal parameters of *T. molitor*

The pupal parameters including the weight, length, duration and survival rate are demonstrated in Table 3. Results of this experiment indicated that all these parameters were significantly affected by the tested diets.

Data in Table 3 showed that the average weight of *T. molitor* pupa was significantly different among all the diets used in this study (P<0.05). The weight of pupae originating from the larvae reared on D-13 was significantly higher (133.53±2.58 mg) followed D-5 with an average weight of 108.04±2.23mgas compared to pupae weight originating from other diets. The pupae recovered from D-12 and D-4 had significantly minimum weight of 77.78 ±1.99 mg and 78.26 ±2.17 mg respectively. It was obvious from the results that heavier and bigger pupae were recorded in diets containing more wheat bran as a feeding substrate.

There was a significant difference in the pupal length of *T. molitor* when fed on diets of various composition (P<0.05) as demonstrated in Table 3. Significantly longest *T. molitor* pupae were recorded in D-13 (17.40±1.14mm) followed by D-5 (15.55± mm) and D-6 (14.13 ±0.25 mm). Shortest pupal length (10.12±0.04 mm) was noted in D-12 which was statistically at par with D-4 (10.34±0.36 mm). It was evident from Fig. 2 (c) that pupal developmental time of *T. miolitor* was also affected by the nature of diets. Significantly shortest duration of pupae (7.36±0.21 days) was observed in D-13 while the longest time was recorded in D-10 (10.41±1.12days) which was statistically similar with D-8 (9.93±0.27 days). The pupal duration in other diets ranged from 8.00 to 9.68 days.

The survival rate of *T. molitor* pupae was greatly affected (P<0.05) by the selected diets and found highest (100%) in D-2, D-7, D-8 while lowest survival rate was observed in D-10 (90.73%) among all the diets. The survival rate of pupae in remaining diets was above >90% (Table 3).

Table 3. Pupal parameters of *T. molitor* fed on different diets under laboratory conditions

Diet	Pupa weight (mg) ± SD	Length (mg) ±SD	Duration (days) ± SD	Survival rate (%) ± SD
D-1	92.59 ±2.63 e	12.29 ±0.31fg	8.26 ±0.10f-h	96.67 ±1.78 bc

D-2	96.21 ±1.46 d	13.17 ±0.86de	9.32 ±0.04b-e	100.00 ±0.00 a
D-3	88.19 ± 2.13 gh	11.48 ±0.17gh	9.68 ±0.35bc	93.33±1.36 de
D-4	78.26 ±2.17 j	10.34 ±0.36i	8.72 ±0.43ef	98.33±1.46 ab
D-5	108.04 ±2.23b	15.55±0.56b	8.00 ±0.17gh	98.33±0.45 ab
D-6	99.25 ±1.34c	14.13 ±0.25c	9.16 ±0.08c-e	91.67±1.81 e
D-7	90.90 ±0.83ef	12.17± 0.49fg	8.74 ±0.12d-f	100.00±0.00 a
D-8	96.79 ± 1.79 d	13.85± 0.41cd	9.92 ±0.27ab	100.00±0.00 a
D-9	89.12 ±0.72fg	12.34± 0.33ef	8.91 ±0.56d-f	98.33±1.59 ab
D-10	84.37 ±2.47i	10.28±0.1253i	10.47 ±1.12a	90.73±3.11 e
D-11	86.56 ±1.03hi	11.20±0.11h	9.66 ±0.38bc	96.38±2.61 bc
D-12	77.78 ±1.99j	10.12± 0.04i	9.43 ±0.29b-d	94.84±1.69 cd
D-13	133.53 ±2.98 a	17.40± 1.14a	7.35±0.21h	98.33±0.67 ab
LSD (0.05)	2.3935	0.8319	0.7035	2.6709

Pupal parameters of *T. moilitor* reared on D_1: RS 20%+ CS 20%+ WB55%+ Y5%, D_2: RS 40%+ CS 40%+ WB15%+ Y5%,D_3: RS 40%+CS 50%+ WB5%+ Y5%,D_4: RS 50%+ CS 40%+ WB5%+Y5%,D_5: RS 40%+ WB55%+Y5%, D_6: CS 40%+ WB55%+Y5% D_7: RS 60%+ WB35%+Y5% D_8: CS 60%+ WB35%+Y5% D_9: RS 80%+ WB15%+Y5%, D_{10}: CS 80%+ WB15%+Y5%, D_{11}: RS 95%+Y5%, D_{12}: CS 95%+Y5%, D_{13}: WB 95%+Y 5%
Means in column followed by different letter are significant at 0.05% level of significance followed by LSD test.

Adult parameters of *T. molitor*

Table 4 demonstrated the average body weight, length, longevity and survival rate of adult of *T. molitor*. It is evident from the results that all these parameters were significantly ($P<0.05$) affected by tested diets under laboratory conditions.

The adult weight of *T. molitor* varied significantly among the feeding diets tested in the experiment ($P<0.05$). According to Table 4, significantly maximum adult weight (113.68±1.32 mg) was recorded in D-13 which was closely followed by D-5 (98.19±1.04mg), whereas minimum weight gain (63.41±1.91 mg) was recorded in D-4 as compared to the other diets.

The body length of adult *T. molitor* was also significantly affected ($P<0.05$) by the type of diets as shown in Table 4. Results indicates that significantly maximum gain in body length of *T. molitor* adult was recorded in D-13 (15.95± 2.17 mm) followed by D-5 ((14.82± 3.05 mm) and D-1 (13.52±1.63 mm), while smallest adults were recorded in D-4 (10.18 ± 1.07 mm) followed by D-12 (11.210±3.11mm), these diets are however significantly different from each other.

Results regarding the longevity of adult mealworms in Table 4 showed significant differences ($P<0.05$) in the longevity of *T. molitor* fed on different diets. Longest adult longevity (88.38±5.22 days) was found in D-2 which was significantly similar with D-3 where the adult lived for a period of 86.38±5.92 days. On the other hand, significantly shortest adult longevity (71.05±2.07 days) and (74.37±3.67 days) were recorded in D-4 and D-5 respectively.

Regarding the percent survival of adult, data clearly stated that maximum survival rate (98.67 ± 1.53%) was recorded in D-13 followed by D-5 (98.12 ± 0.18%), D-9 (97.24 ± 1.65), D-7 (96.95 ± 1.62), D-11(96.91 ±0.52) and D-10 (96.03 ±1.69) which were all statistically at par. A significantly lower survival rate (93.410±1.29%) and(93.78±1.77%) was recorded in D-2 and D-3 respectively, Table 4.

Table 4. Adult parameters of *T. molitor* fed on different diets under laboratory conditions

Diet	Weight (mg)± SD	Length (mm)± SD	Longevity (days)± SD	Survival rate (%)± SD
D-1	88.820 ± 1.45c	13.52±1.63c	77.58±3.45e-g	95.59± 1.06c-f
D-2	75.063 ±0.36h	12.80±3.08de	88.37±5.22a	93.410 ± 1.29f
D-3	77.457 ±1.02fg	12.20 ±2.11ef	86.38±5.92ab	93.78 ± 1.77ef
D-4	63.410 ±1.91i	10.18 ±1.07h	71.05±2.07h	94.21 ± 1.69ef
D-5	98.193 ±1.04b	14.82 ±3.05b	74.37±3.67gh	98.12 ± 0.18ab
D-6	86.587 ±0.88d	13.26 ±2.04cd	81.31±4.04c-e	94.95 ± 1.47c-f
D-7	83.220 ±0.93e	12.54 ±3.06ef	79.38±3.85d-f	96.95 ± 1.62a-d
D-8	79.127 ±1.51f	12.09 ±2.12f	84.64±2.01a-c	95.18 ±1.81c-f
D-9	83.393 ±1.08e	13.18±3.07cd	83.90±3.42a-d	97.24 ± 1.65a-c
D-10	77.172 ±0.82g	12.70±3.10de	79.50±4.22d-f	96.03 ±1.69b-e
D-11	79.304 ±0.77f	12.78 ±2.16de	81.87±5.63b-e	96.91 ±0.52a-d
D-12	73.962 ±1.22h	11.21±3.11 g	80.08±4.95c-e	94.58 ± 1.09d-f
D-13	113.68 ±1.32a	15.95± 2.17 a	75.19±3.93f-h	98.67 ± 1.53a
LSD (0.05)	1.9528	0.6009	4.7757	2.3865

Adult parameters of *T. moilitor* reared on D_1: RS 20%+ CS 20%+ WB55%+ Y5%, **D_2**: RS 40%+ CS 40%+ WB15%+ Y5%,**D_3**: RS 40%+CS 50%+ WB5%+ Y5%,**D_4**: RS 50%+ CS 40%+ WB5%+Y5%,**D_5**: RS 40%+ WB55%+Y5%, **D_6**: CS 40%+ WB55%+Y5% **D_7**: RS 60%+ WB35%+Y5% **D_8**: CS 60%+ WB35%+Y5% **D_9**: RS 80%+ WB15%+Y5%, **D_{10}**: CS 80%+ WB15%+Y5%, **D_{11}**: RS 95%+Y5%, **D_{12}**: CS 95%+Y5%, **D_{13}**: WB 95%+Y 5%
Means in column followed by different letter are significant at 0.05% level of significance followed by LSD test.

Oviposition of *T. molitor*

The female fecundity, means number of eggs per day and oviposition period of female *T. molitor* are presented in Table 5. Diets had significant effects on the eggs production of *T.*

molitor (P<0.05). Results revealed that fecundity of female *T. molitor* was significantly higher in D-13 (272.99±5.0 eggs) followed by D-5 where the mean number of eggs laid by a single female were 197.14± 1.66 while significantly lower female fecundity was observed in D-11 (109.84± 1.62) and D-12 (117.14±2.43). The total number of eggs deposited by a female in D-2, D-3, D-7 and D-10 was non-significant in comparison to the overall fecundity of females in other diets.

Significant variation (P=0.000) was recorded in the number of eggs laid per female fed on different diets Table 5. Significantly maximum number of eggs per female per day ((9.71±2.29) were observed in D-13followed by D-5and D-1 where the female deposit (5.18 ±2.3) and (4.31±2.05) eggs/day respectively. However, significantly minimum number of eggs (2.27±1.14) were laid by each female per day in D-11.

The oviposition period of female *T. molitor* varied significantly (P=0.000) among the diets maintained at 26+1 C, 65% R.H under laboratory conditions. The diet quality greatly affected the oviposition duration of the female. According to Table 5, longest oviposition period of female was recorded in D-10 (60.027 ±2.13days) followed by D-3 (51.10 ±0.96 days), while oviposition period was significantly shortest in D-13 where the female deposit her eggs for about 28.67±2.08 days.

Table 5. Oviposition of *T. molitor* adults fed on different diets under laboratory conditions

Diets	Total Fecundity ± SD	Average Fecundity (female day-^{1})± SD	Oviposition period± SD
D-1	181.61 ± 1.44c	4.31±2.05c	42.02 ±1.89gh
D-2	166.86 ± 4.51e	3.79±2.04e	43.14± 2.08fg
D-3	172.93 ± 4.84de	3.37±2.15fg	51.10±0.96 b
D-4	148.39 ± 2.09g	3.29±2.05gh	45.07 ±1.71ef
D-5	197.14 ± 1.66b	5.18±2.3b	38.67 ±2.08i
D-6	159.74 ± 4.52f	3.53±1.13f	45.57 ± 1.47def
D-7	169.02 ± 1.43de	4.23±1.21c	40.17 ±0.88hi
D-8	174.84 ± 4.33cd	4.05±1.13d	43.02±0.48fg
D-9	155.09 ± 9.41fg	3.16±1.05h	49.33 ±1.53bc
D-10	168.63 ± 1.62de	2.80±2.23i	60.03 ±2.13a
D-11	109.84 ± 1.62i	2.27±1.14k	48.11 ±1.05cd
D-12	117.14 ± 2.43h	2.54±1.27j	46.06 ±0.85de
D-13	272.99 ± 5.00a	9.71±2.29a	28.67 ±2.08 j
LSD (0.05)	6.8966	0.1654	2.6434
***T. moilitor* female oviposition on D_1**: RS 20%+ CS 20%+ WB55%+ Y5%, **D_2**: RS 40%+ CS 40%+ WB15%+			

Y5%,**D$_3$**: RS 40%+CS 50%+ WB5%+ Y5%,**D$_4$**: RS 50%+ CS 40%+ WB5%+Y5%,**D$_5$**: RS 40%+ WB55%+Y5%, **D$_6$**: CS 40%+ WB55%+Y5% **D$_7$**: RS 60%+ WB35%+Y5% **D$_8$**: CS 60%+ WB35%+Y5% **D$_9$**: RS 80%+ WB15%+Y5%, **D$_{10}$**: CS 80%+ WB15%+Y5%, **D$_{11}$**: RS 95%+Y5%, **D$_{12}$**: CS 95%+Y5%, **D$_{13}$**: WB 95%+Y 5%
Means in column followed by different letter are significant at 0.05% level of significance followed by LSD test.

Proximate compositions of different diets used for *T. molitor* rearing under laboratory conditions

Proximate compositions of different diets used as a feeding substrate for mealworms rearing in this experiment are presented in Table 6. The highest % moisture content was recorded in D-13 while lowest was recorded in D-11 and D-9. Maximum crude protein were recorded in D-13 (17.79±0.10%) closely followed by D-5 (15.25±0.28%) while minimum protein contents were recorded in D-12 (6.37±0.06%). Results regarding the crude fats indicated that D-8 contained the highest fat contents (4.33±0.05&) while lowest fats were found in D-3 (1.36±0.07%) among all the diets. Significantly highest crude fiber (38.44±0.40%) and ash contents (12.26±0.06%) were observed in D-4. Minimum %NFE (42.34±0.25%) was detected in D-4 while maximum % NFE (59.06±0.21%) and (58.01±0.09%) were recorded in D-13 and D-1 respectively on dry matter basis which are statistically at par with each other.

Table 6. Proximate compositions of different diets used for *T. molitor* rearing under laboratory condition

Diets	Moisture %±SD	Protein (%)±SD	Fats (%) ± SD	Fiber (%)±SD	Ash (%)±SD	NFE (%) ±SD
D-1	9.91±0.14de	13.45 ±0.54c	3.84±0.09c	16.73±0.39i	8.4±0.05g	58.01±0.09a
D-2	9.60±0.37e	7.74±0.36h	2.81±0.10f	30.93±1.04d	9.31±0.15e	49.83±1.20d
D-3	10.62±0.25c	6.79±0.18i	1.36±0.07j	35.31±.039b	8.6±0.11f	48.42±0.09ef
D-4	8.82±0.09f	6.46±0.15i	3.35±0.08d	38.44±0.40a	12.26±0.06a	42.34±0.25h
D-5	9.90±0.24de	15.25±0.28b	4.13±0.13b	19.85±0.64h	7.23±0.05i	54.02±0.12b
D-6	9.84±0.17de	12.54±0.12d	3.92±0.07c	20.61±0.67h	4.55±0.06l	58.54±0.15a
D-7	11.02±0.24b	11.49±0.23e	2.12±0.10h	22.13±0.13g	11.93±0.09b	53.10±0.05b
D-8	11.93±0.10a	10.8±0.43f	4.33±0.05a	27.3±0.11f	4.63±0.05l	53.54±0.47b
D-9	8.79±0.13f	8.25±0.18g	3.16±0.13e	29.77±0.08e	10.25±0.04d	47.17±0.18g
D-10	9.99±0.15d	8.45±0.26g	2.49±0.09g	32.38±0.52c	5.65±0.12j	51.37±2.16c
D-11	8.74±0.19f	7.36±0.03h	2.41±0.08g	31.62±0.43cd	10.73±0.12c	47.97±0.08fg
D-12	8.58±0.18f	6.37±0.06i	1.91±0.03i	35.36±0.10b	7.49±0.06h	49.34±0.77de
D-13	11.80±0.13a	17.79±0.10a	2.08±0.05h	16.42±0.03i	5.16±0.07k	59.06±0.21a
LSD (0.05)	0.3260	0.4429	0.1215	0.7933	0.1276	1.2391

Feeding diets were **D$_1$**: RS 20%+ CS 20%+ WB55%+ Y5%, **D$_2$**: RS 40%+ CS 40%+ WB15%+ Y5%,**D$_3$**: RS 40%+CS 50%+ WB5%+ Y5%,**D$_4$**: RS 50%+ CS 40%+ WB5%+Y5%,**D$_5$**: RS 40%+ WB55%+Y5%, **D$_6$**: CS 40%+ WB55%+Y5% **D$_7$**: RS 60%+ WB35%+Y5% **D$_8$**: CS 60%+ WB35%+Y5% **D$_9$**: RS 80%+ WB15%+Y5%, **D$_{10}$**: CS 80%+ WB15%+Y5%, **D$_{11}$**: RS 95%+Y5%, **D$_{12}$**: CS 95%+Y5%, **D$_{13}$**: WB 95%+Y 5%
Means in column followed by different letter are significant at 0.05% level of significance followed by LSDtest.

Proximate composition of *T. molitor* larvae fed on different diets under laboratory conditions

Table 7 showed the proximate composition of *T. molitor* larvae reared on thirteen different diets. The proximate composition (ash, fats, fiber, protein contents) of the larvae were greatly influenced by the composition of diets. The crude proteins were significantly higher in the larvae reared on D-13 (57.21±0.91%) whereas lowest protein contents were recorded in D-12 (37.88±1.11%) and D-8 (38.15±0.87%) which are non-significant. On other hand least amount of crude fats (19.27±0.75%) and ash contents (3.04±0.13%) were recorded in D-13 but highest % fats (34.02±0.99%) and ash (7.92±0.17%) were recorded in D-12 and D-8 respectively. In addition, the % crude fibers were maximum in larvae fed on D-4 (12.27±0.49ab), D-10 (13.01 ±0.05) and D-12 (13.13±0.29) although the fiber contents of these larvae significantly at par with each other. Diet 4 and 6 showed significantly higher %NFE in comparison to rest of tested diets. The % moisture of larvae ranged between 59.25-64.52%.

Table 7. Proximate composition of *T. molitor* larvae fed on different diets under laboratory conditions

Diets	Moisture (%) ± SD	Protein (%)±SD	Fats (%) ± SD	Fiber (%)±SD	Ash (%)±SD	NFE (%)±SD
D-1	62.19 ±0.92cd	45.61±0.84 c	27.43± 0.47 ef	7.73±0.26 h	7.57±0.25b	11.66±0.30d
D-2	63.79 ±0.18ab	42.76±0.91 e	30.15±0.86 bc	10.39±0.62 ef	6.33±0.16e	10.37±0.22e
D-3	61.29 ±0.32de	37.12±1.01 g	33.17±1.06a	9.29±0.83fg	7.82±0.04ab	12.6±0.11c
D-4	59.73 ±0.49f	39.76±0.37 f	29.36±1.05cd	12.27±0.49ab	5.53±0.50f	13.08±0.13a
D-5	62.33 ±1.21b-d	48.33±0.60 b	24.58±0.74g	11.61±0.64 b-d	7.13±0.08c	8.35±0.10g
D-6	59.76 ±0.80f	43.17±0.71 de	28.17±0.14de	10.97±1.01c-e	4.68±0.11h	13.01±0.03ab
D-7	64.52 ±0.67a	44.39±0.96 cd	26.13±1.11fg	8.95±0.13gh	7.86±0.13ab	12.67±0.38bc
D-8	61.5 ±1.17c-e	38.15±0.87 g	33.16±1.95a	11.69±0.19 b-d	7.92±0.17a	9.08±0.08f
D-9	60.45 ±0.82ef	42.87±.031 e	31.21±1.16b	10.56±0.95de	6.67±0.09d	8.84±0.51f
D-10	59.52 ±1.03f	40.61±1.26 f	33.57±1.45a	13.01 ±0.05a	6.54±0.05de	6.64±0.16h
D-11	63.82 ±1.03bc	40.21±0.66 f	33.22±1.15a	11.86±0.92bc	6.32±0.05e	8.79±0.12f
D-12	61.59 ±1.40c-e	37.88±1.11g	34.02±0.99a	13.13±0.29a	5.21±0.03g	10.2±0.08e
D-13	62.44 ±0.91b-d	57.21±0.91a	19.27±0.75h	11.78±1.19bc	3.04±0.13i	9.04±0.09f
LSD (0.05)	1.5193	1.4082	1.8073	1.1476	0.3041	0.3695

Larvae were reared on D_1: RS 20%+ CS 20%+ WB55%+ Y5%, **D_2**: RS 40%+ CS 40%+ WB15%+ Y5%,**D_3**: RS 40%+CS50%+ WB5%+ Y5%,**D_4**: RS 50%+ CS 40%+ WB5%+Y5%,**D_5**: RS 40%+ WB55%+Y5%, **D_6**: CS 40%+ WB55%+Y5% **D_7**: RS60%+ WB35%+Y5% **D_8**: CS 60%+ WB35%+Y5% **D_9**: RS 80%+ WB15%+Y5%, **D_{10}**: CS 80%+ WB15%+Y5%, **D_{11}**: RS95%+Y5%, **D_{12}**: CS 95%+Y5%, **D_{13}**: WB 95%+Y 5%
Means in column followed by different letter are significant at 0.05% level of significance followed by LSDtest.

DISCUSSION:

Growth and performance of insects are greatly dependent on nature of diets. An effective diet plays a great role in mass production of beneficial insects. Diets significantly affects the larval weight of *T. molitor* in our study. Similar findings were made by Ahmad *et al.* (2013) and Xu *et al.* (2010) that the food directly affects the larval and pupal duration along with weight and length of larvae and pupae. Some earlier studies of Zhang *et al.* (2019) confirm these findings by rearing mealworms on diets comprised of wheat bran, corn stover, soybean meal and distiller grain. During the study they found significant differences among the larval weight fed upon the various diets. On wheat bran highest weight gain was recorded, whereas the lowest weight gain was recorded in corn stover. Similarly, in our result we also observed minimum weight gain in diets contain corn straw. Saadiya and Defrawy (2022) also reported that larval length varied significantly reared on different diets, the longest larva was recorded in wheat bran and shortest in corn stover.

Addition of 5% yeast to wheat bran and other diets has positive impact on the growth of larva and improve length and weight gain. These results are in conformity with the findings of Kim *et al.* (2016). They observed that mealworm raised on diet containing yeast resulted high weights than other diets. More than 95% survival rate was recorded in wheat bran supplement diets in the current studies. Zim *et al.* (2022) and Saadiya and Defrawy (2022) studied survival rates of mealworms on numerous diets and reported highest survival rate in diets mixed with wheat bran. Oonincx *et al.* (2015) and Van Broekhoven *et al.* (2015) experimented improvements in the survival and larval developmental time when additional ingredients were added to the wheat bran.

The longest developmental time of larvae was recorded in D-10 while the shortest duration was noted when fed on D-13. Similar observations were made Kim *et al.* (2014), they reported shortest developmental time of larvae fed on wheat bran while longest was recorded other tested diets composed of agricultural wastes. This might be due to the high protein contents in diets which speed up the development of mealworms. These findings are also endorsed by Van Broekhoven *et al.* (2015), they monitored longest developmental time on diets containing low protein as compared to the protein rich diets. According to Jensen *et al.* (2017) the insect grow faster when fed with high protein containing diets. Other studies conducted by Rumbos *et al.* (2020) also reported faster growth rate of *T. molitor* on high protein diets especially derived from the yeast. Mealworms may obtain all the required nutrients for their growth, development and reproduction from wheat bran as reported by Ortiz *et al.* (2016).Morales-Ramos *et al.* (2010) also reported increased growth rate of *T. molitor* on high protein diets especially derived from the yeast. Some diets showed lower larval length, weight, duration and survival in the current studies. The reasons could be preferences and digestibility of the diets by the larvae. These results are at par with the findings of Rumbos *et al.* (2020) who also stated that lower digestibility of diets might results in lower larval parameters.

Pupal weight was greatly dependent on larvae especially fed on wheat bran containing diets in this experiment. Kim *et al.* (2017) also observed heaviest pupa and highest survival rate in wheat bran. In another study done by Kim *et al.* (2019) further strengthens our finding by recording high weight and survival rate in *T. molitor* pupa reared on wheat bran compared to other diets. Park *et al.* (2012) reared the mealworms on two different diets (wheat bran and swine feed) and found significantly higher pupal weight and survival rate in wheat bran fed treatments. Kim *et al.* (2016) also reported heavier pupae recovered from wheat bran as compared to the other diets combinations.

Very few studies are available on the oviposition and adult performance on different diets. Most of the studies were focused on larval development and their nutritional composition. In the current studies a comprehensive list of diets was evaluated for the oviposition, development and adult performance. Oviposition was also affected by the nature of diet as well as adult weight. More eggs were witnessed in diets supplemented with higher amount of wheat bran. This might be due to the higher protein content in the diets which enhances the egg production. The studies of Zhang *et al.* (2019) reported wheat bran to have higher protein contents as compared to other diets which agree with the current findings. The results are also validated by Urrejola *et al.* (2011), Park *et al.* (2012) and Morales-Ramos *et al. (*2012) who reported more eggs deposited by the females fed on different diets supplemented with wheat bran. Another reason regarding the low number of eggs laid by *T. molitor* fed rice straw95%+yeast5% and corn straw95%+yeast5%, might be due to the lower growth rate and development of larvae, pupae and adults as reported by Nukmal *et al.* (2018). In this study it was observed that the pupal and adult weight of the mealworms have significant effect on the fecundity of *T. molitor*. Similar observations were made by Park *et al.* (2012) and Morales-Ramos *et al.* (2012) who also revealed that fecundity of *T. molitor* is greatly dependent on the pupal as well as adult weight.

Crude protein (CP) varied among the diets. The diet 13 has highest %CP and larvae reared upon this diet showed significantly high larval length and weight in the current study. Earlier studies of Morales Ramos *et al.* (2012) also confirm that those diets which contain high protein showed great impact on larval length and weight. Ruschioni *et al.* (2020) noted crude protein ranged from 13.19-16.84 in different diets. In our study the values of protein are slightly different ranging from 6.73-17.79. This might be due to the combination of the tested diets as we mainly fed the larvae on byproducts of wheat bran. Bumroongsook and

Nahuanong (2018) verified a total of 18% crude protein, 3.6% fats and 9.26 % fibers in wheat bran, which is very close to our observation in the current study as we recorded 17.79% CP, 2.08% fats and slight higher 16.42% fiber in wheat bran and yeast. The NFE of our experiment ranged from 42.3 to 59.06%, comparatively lower than those reported by Ruschioni *et al.* (2020).

The quality of diet has a large impact on the proximate composition of mealworm larvae where the high protein content of diet resulted in a high dry matter basis crude protein in the mealworm larvae as described by Heckmann *et al.* (2018). We observed maximum % crude protein contents (57.21%) in larvae fed on 95% wheat bran and 5 % yeast. However, Ravzanaadii *et al.* (2012) detected (46.44% % CP) in larvae raised on wheat bran only. The highest CP in our results might be due to the addition of % 5 yeast in the wheat bran which was endorsed by Kim *et al.* (2017) that addition of yeast to diets increases the larvae growth and performance. Our results are in line with the findings of Ghaly and Alkoaik (2009), according to their results larvae attain the highest protein when fed on diet composed of wheat flour (95%) and Yeast (5%). In D-5 (Rice Straw 40% and 55% wheat bran and 5% yeast) the total recorded CP was 48.33% in the study. This percentage is very near to the findings of Jajic *et al.* (2022). They reared mealworm on different feed stuff and found highest CP on wheat bran followed by mixture of cereal such oat and barely 48.2%. Crude Protein in larvae reared on D-1, D-2, D-6, D-7 and D-9 ranged between 42.7 to 45.61%. Similar conclusions were also recorded by earlier studies of Alves *et al.* (2016). They studied the CP content on different combination of flour mixture of wheat, soya bean and bocaiuva pulp and found 44.8% crude protein. Similarly, our results indicated that feeding mealworm larvae on protein rich diets such as wheat bran alone and mixes with rice straw in different percentage resulted in high level of protein in larvae as reported by Oonincx *et al.* (2015). Siemianowska *et al.* (2013) reported 44.72% CP in oat flakes which is also in the range of our findings. Lowest %CP was noted in D-3 and D-12 which were 37.12% and 37.88% respectively. The observed values percent CP by Ruschioni *et al.* (2020) and Tan *et al*. (2018) were also close to the current findings in D-3 and D-12, however the composition of the diets was different from their diets.

Conclusion and Recommendations:

Diet played an important role in the development and nutritional profile of the *T. molitor*. Among the tested diets, wheat bran rich based diets, D-13 (95% Wheat bran + 5%Yeast) and D-5 (40% Rice straw + 55% Wheat bran+5% yeast) were found the most promising diets that accelerate and promote fecundity, larval growth and development of *T. molitor* and highest protein, crude fat content and is therefore, suggested for mass prodcution of *T. molitor*.

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Author's contribution:

Fazli Amin (FA) and Amjad Usman (AU) designed, conducted the experiment, analyzed the data and wrote the draft. AU reviewed the manuscript and provides valuable suggestions for improvement.

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