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EFFECT OF DIFFERENT DIETS ON SOME LARVAL BODY CHARACTERISTICS AND FOOD UTILIZATION EFFICIENCY OF TENEBRIO MOLITOR (COLEOPTERA: TENEBRIONIDAE)

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ABSTRACT: In the present study, nutritional composition and growth parameters of yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae were evaluated after 30 days of feeding using different food materials i.e. Wheat as (diet 1) , oats (diet 2) , chicken 21% (diet 3)- corn Stover (diet 4) , oats + wheat bran (diet 5) , oats + corn stover (diet 6) , oats + chicken feed 21% (diet 7) , wheat + corn stover (diet 8), wheat bran + chicken feed 21% (diet 9) , chicken feed 21% + corn stover (diet 10)- and the last diet is a mixture of the previous four ingredients (wheat bran + corn stover + chicken feed 21% + oats (diet 11). For growth parameters, yellow mealworm larvae fed on Wheat as (diet 1) – oats (diet 2) – chicken feed 21 % and mixture of them have the highest values in specific growth rate and feed conversion efficiency .Nutritional analysis showed significant differences of contents between tested diets; Where the analysis showed the highest percentage of protein and fat when the larvae fed Diet 3 and 1 On the other hand ,highest level of crude fiber occurred when larvae fed on corn Stover and wheat + corn stover . In general, yellow mealworm mass rearing using Wheat، oats, chicken feed 21 % and mixture of them showed a good result on growth rate and nutritional composition.

Key words: Different diets, larval body characteristics, food utilization efficiency, *Tenebrio molitor.*

INTRODUCTION

As a result of climatic change, which in turn led to a significant shortage of food at the global level, this led to the tendency to use some new alternatives in food, for example, the use of insects in food for humans, directly or indirectly, as they are used as feed for poultry, fish, and animals. This is because these insects can convert food that is poor in proteins in their body to a high content in proteins and that is better than other animals, and therefore they can be relied upon as a rich source of protein, either for humans as direct food (Joensuu 2017) or incorporated into the most familiar components of poultry, fish and farm animals. Therefore, it is used as indirect food for humans. There are types of insects used for the previous purposes, including wax worms, locusts, black soldier fly, and yellow bran worm, as it was found that these types of insects feed on a diet poor in protein content and convert it in their bodies to a high protein content (Ng WK *et al.* 2002).

Yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) is the most promising insect species for industrial utilization and commercial large-scale production. Larvae are used as food for fish, reptiles, turtles, birds and small pets that are raised at home (Huis 2013 and Huis *et al*. 2013), and these larvae are raised all over the world for the previous purposes (Morales and Rojas, 2015, Morales *et al* . 2010 , 2012 , and 2015), as they are considered the best food that contains high levels of protein and fat. Recently, the larvae of this insect have been included in the list of insects allowed to be used as ingredients in some animal feed in the European Union (Panini *et al*. 2017). In addition, it was evaluated as a component of feed ingredients for poultry and fish and gave excellent results. (FAO 2014, Nagasawa and Cruz 2004 , Shakouri and Yazdi 2012, Ng WK *et al*. 2001, Marco *et al*. 2015). This insect has attracted the attention of many researchers to use it as food for humans in a direct way. This interest has increased through the development of food systems in what is known as the insect breeding industry, in order to produce larvae commercially, especially in Western countries

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Morales *et al.* 2012 and Siemianowska *et al.* 2013). Newly, yellow mealworm, *T. molitor* has been used as human food in some European and Asian countries. (Nagasawa and Cruz 2004. Shakouri and Yazdi 2012, Siemianowska *et al.*2013)

Life cycle, feeding Habitats and Distribution of Yellow mealworm :

Yellow mealworm life cycle includes Egg, larva, pupa and adult. The average number of deposed eggs for female is 400-500 eggs. The adult laid bean shaped sticky eggs and shiny white in color, these is singly or in clusters in the food. The incubation period of eggs is about 2 weeks at 25-27°C, after that, the eggs hatch into larvae about 2 mm in length, which are initially white in color, then turn to bright yellowish brown after feeding. The larva elongate and cylinder, it has 6 small legs behind the head directly, and there is a pair of short horns on the edges of the abdomen. The period of larval stage is about 6-8 months at optimum conditions; this period may extend to two years when the environmental conditions are not suitable. At the end of this period, the length of the larva reaches 2.0 – 2.5 cm, mature larvae turn into pupae about 1 cm long and yellowish brown.in color, the period of pupa takes from 6 to 18 days, depending on the temperature. The adult emergence occurs at the end of the pupal stage, and they are initially shiny brown in color and then turn black when they are sexually mature. After about 3 days, they are ready to meeting process. The female mates several times during its lifespan, which ranges from 37 to 96 days. The yellow mealworm *T. molitor* is spread all over the world. The larvae of this insect are present in the winter, and the pupae are formed in the spring and early summer. The adults are present in the summer where they mate, lay eggs and it continues their life cycle (Ghaly and Alkoaik 2009).

This insect is considered of mmoderate importance, infecting all stored grains and their products. This insect prefers ground and decaying grains, which have increased moisture content. It can also feed on flour, bran, grains, bread, mill wastes, meat waste, feathers, dead insects, moulting skins 23. The insect is found in dark, damp places, feed bags, chicken droppings, and bird breeding places. (Morales and Rojas, 2015 , Morales *et al*. 2010 and 2015).

As a result of little information on the suitable nutrition for the yellow mealworm (*Tenebrio molitor*), the aim of this study was to evaluate some food diets and their effect on the growth characteristics and nutritional composition of the larvae, to mass rearing on a large scale and commercially uses for human and animal feeding purposes.

Materials and methods

To evaluate the suitable nutrition for the mealworm *Tenebrio molitor* thirty second instar larvae were fed on 20 grams of 11 diets: Wheat as (diet 1) , oats (diet 2) , chicken 21% (diet 3) which consists of: yellow corn - soybean meal (44%) - corn gluten (60%) - di-calcium phosphate - limestone - table salt - a mixture of vitamins and minerals - anti-caking - sodium bicarbonate - choline chloride 60% - L. Lysine Hydrochloride 98,5% - Methionine Hydroxy Analog Calcium (Produced by New Feed Company), corn Stover (diet 4) , oats + wheat bran (diet 5) , oats + corn stover (diet 6) , oats + chicken feed 21% (diet 7) , wheat + corn stover (diet 8), wheat bran + chicken feed 21% (diet 9) , chicken feed 21% + corn stover (diet 10) and the last diet is a mixture of the previous four ingredients (wheat bran + corn stover + chicken feed 21% + oats (diet 11).

Larvae were fed to the previous environments in equal proportions for each diet item (1:1), and the total weight of the diet environment was 20 grams. The larvae were weighed at the beginning and end of the experiment, and the lengths and weights of both larvae and pupae were taken. The feeding period was three months at 27°C±2 and 65%±2 starting with the second instar. The diets were changed daily with the same amounts used in the experiments.

Evaluation of growth performance and survival of *Tenebrio molitor*:

Feed conversion, growth performance and survival rate in each treatment group were determined after feeding trial. Performance in growth and feed utilization were determined based on the criteria: length gain (LG), weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR) and food conversion efficiency (FCE) (Oonincx *et al.* 2015 and Agbo *et al.* 2011). Calculation of each criterion was done based on the formulas below:

a. LG (%)=100[(Final length−Initial length)/Initial length].
b. WG (%)=100[(Final weight− Initial weigh)/Initial weight].
c. SGR (%)=100[(ln Final weight of alive larvae)–(ln Initial weight or larvae) /total feeding days].
d.FCR (g/g)=Weight of ingested diet/Weight gained of alive mealworms.
e. FCE (%)=100(Weight gained of alive mealworms /Weight of ingested diet).
f. Survival rate (%)=100(Initial number of mealworms–Number of dead mealworms)/Initial number of mealworms.

Nutrition analysis: The moisture, ash, protein and fat contents of mealworms representing a full range of weights (4.8-182.7 mg) were carried out. Moisture content: The moisture content was determined gravimetrically using 50 live worms (5 from each weight group). The oven dry method procedure described in APHA (1990) was followed.

Statistical analysis: Statistical analyses were conducted using SPSS Statistical Package (2010). Data were subjected to one-way ANOVA analysis and Dunkin Multiple Comparison Test were evaluate differences between groups with the acceptance at p<0.05.

RESULTS AND DISCUSSION

Effect of different diets on some body characteristics:

1- Larvae

The food type has a clear effect on the weight of the larvae after the treatment period. The mean gain weights of *T. Molitor* larvae fed on different diets were summarized in Table (1). Mealworms *T. Molitor* larvae fed on Diet 1 showed the highest gain weight while the lowest weight was found in *T. Molitor* larvae fed on Diet 4. Generally, larvae fed on the tested diets significantly differ from each other.

Table (1): Effect of different diets on mean weight of larvae and pupae and mean length of larvae

Different Diets	Larva						Resulted pupa
	Initial number	Mean initial weight (mg)	Final number of alive larvae	Mean final weight(mg) of alive larvae	mean initial length (cm)	Mean final length(cm)	Mean weight (mg)
Diet (1)	90	0.03±0.01	90	0.18±0.3a	1.5±0.71	2.8±0.31a	0.18±0.51a
Diet (2)	90	0.03±0.02	90	0.15±0.3b	1.4±0.32	2.5±0.44b	0.15±0.21b
Diet (3)	90	0.03±0.04	90	0.16±0.2b	1.5±0.51	2.7±0.56a	0.15±0.11b
Diet (4)	90	0.04±0.03	79	0.10±0.7d	1.5±0.43	1.9±0.32d	0.11±0.10c
Diet (5)	90	0.03±0.02	88	0.18±0.4a	1.3±0.81	2.5±0.11b	0.16±0.31a
Diet (6)	90	0.04±0.06	87	0.12±0.1c	1.4±0.72	2.4±0.64bc	0.12±0.01c
Diet (7)	90	0.04±0.04	90	0.17±0.5a	1.4±0.62	2.6±o.43a	0.14±0.13b
Diet (8)	90	0.03±0.01	88	0.14±0.8bc	1.3±0.80	2.5±0.71b	0.13±0.12bc
Diet (9)	90	0.04±0.02	89	0.18±0.4a	1.3±0.30	2.6±0.62a	0.16±0.14a
Diet (10)	90	0.04±0.07	89	0.13±0.3c	1.3±0.21	2.3±0.55c	0.13±0.10bc
Diet (11)	90	0.04±0.09	89	0.14±0.1bc	1.5±0.83	2.5±0.42b	0.14±0.13b
P value	-	-	-	0.00**	-	0.000***	0.00**
LSD	-	-	-	0.02	-	0.11	0.01

Means followed by same letter in column are not significantly different at 5% level.

Also, the effect of food type has a significant effect on the gain length of *T. Molitor* larvae. Results in Table (1) showed the significant effect of different diets on the length of tested larvae where it was found that larvae fed on the diet 1 led to a significant increase in the length of the larvae at the end of the experiment period, the highest length was 2.8 cm. Different diets had significantly effect on the final length at the end of experiment. The lowest length was occurred in the diet 4, the length, the mean length of larva was 1.9 cm. Generally, tested diets significantly effect on the length of tested larvae (Figure 1). Also, the survival rate of tested larvae were differed significantly. Diet 1 showed the highest survival rate followed by Diet 2 (Figure 2).

a) Larval body length after feeding on wheat bran, chicken feed 21% and oats starting from the left, respectively.

b) Larval body length after feeding on wheat bran and corn stover, respectively.

c) Larval body length after feeding on (oats + chicken feed 21%) - (wheat bran + chicken feed 21%) - (wheat bran + corn stover) - (corn stover)- (chicken feed 21% + corn stover) – (oats + corn stover) , respectively.

Fig. (1): Effect of tested diets on larval length of *T. Molitor*

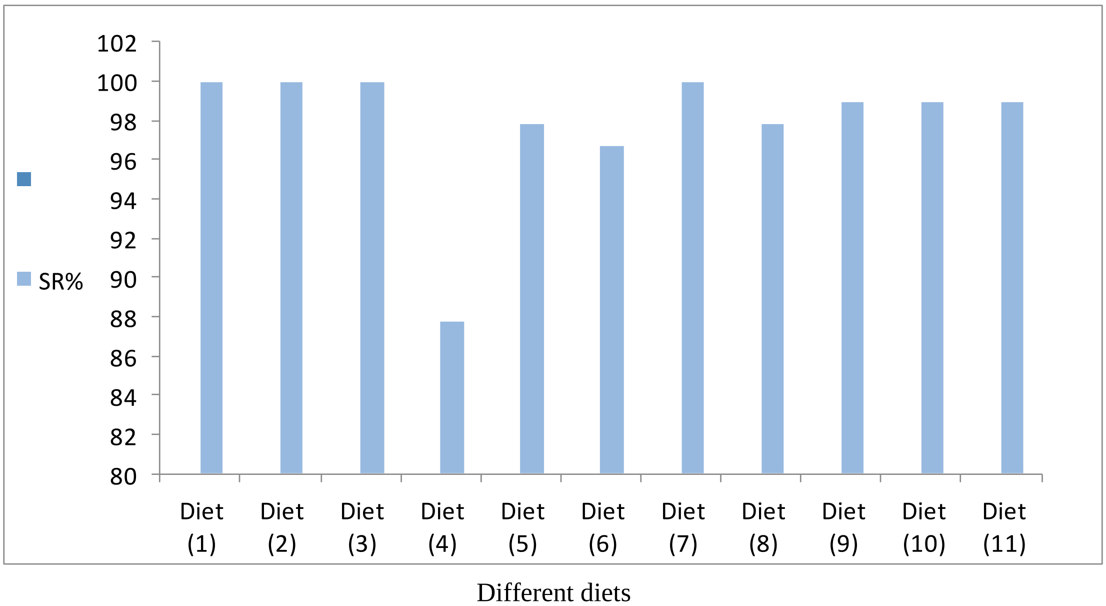

Fig (2) : Effect of different diets on Survival rate % of larvae .

2- Pupae

Also for the pupae, the results were going in the same trend, as it was found that the type of food affects the weight of the pupae, and this is what the results show in Table (1).

The growth parameters and food conversion of *T. Molitor* were showed in (Table 2). *T. Molitor* larvae fed on Diet 1 showed longest body's length (LG) gain (93.33%), followed by Diet 3 (86.67%) but the lowest LG % was occurred when larvae fed on Diet 4 (26.67 %), there was a significant difference among tested diets (p>0.05) (Table 2).

Likewise, the same trend was noticed on the weight-gained (WG) and specific growth rate (SGR); the larvae fed on Diet 1 showed the highest WG value at 125 % and the lowest WG occurred when the larvae fed on Diet 4 (66.67 %).

About SGR values obtained data showed that the highest values occurred when the larvae fed on Diet 1 and 9 at (1.17 %) but the lowest SGR value was found with Diet 4 at (0.74 %).

As for the efficiency of food conversion value (FCR), *T. Molitor* larvae fed on Diet 4 , 6 and 11 showed the highest food conversion ratio among the other tested diets with 13.15, 13.20 and 14.28 % , respectively. On the other hand, *T. Molitor* fed on Diet 3 and 10 showed the lowest FCR value at 9.5 and 8.23 %, respectively (Table 2). In fact, FCR value always related to the protein content in diet. Higher protein content in the diet helps in muscle growth and formation, and thus led to a lower FCR; and vice versa (Van *et al.* 2015).

Data in Table (2) showed that the food conversion efficiency values for tested diets were differed significantly. *T. Molitor* larvae fed on Diet 10 have the highest food conversion efficiency (FCE) at 12.16 % among the tested diets followed by 2. 9. 8. 1, 7 and 3, but the lowest values were occurred when larvae fed on Diet 4 and 11 with 7.41 and 7.58 %, respectively (Table 2). The growth indicators and FCE were highest when using Diet 1, and there were similar results for the Diets 3, 9 and 10, it showed highest survival rate in *T. Molitor* cultivation (Fig. 1).

Data in Table 3 showed that Diet 1 was the better food in terms of nutrients e.g. crude protein, moisture, ash and crude fat. It was clear from Table (3) that the percentage of crude protein was high when the larvae fed on the Diet 1, 2 and 3, there was no significant difference, while the percentage of protein decreased to some extent when the larvae fed on the Diets 4 and 8. It was clear that the lowest percentage of protein appeared when the larvae fed on the Diet 4. As well as the percentage of crude fat, it became clear from this study that when the percentage of crude protein was increased, the percentage of crude fat decreased as shown in Table 3 (Shu, 2018), Higher protein content helps in muscle development (Giroux, 1996), and thus lowering FCR; and vice versa (Van *et al.* 2015).Also, diet with high crude protein and crude fat could produce longest larvae at the 14th instar's stage (Morales et al. 2012 , Huis *et al.* 2013 and Siemianowska *et al.*2013) The results showed that there was a significant difference between the tested diets, but the highest percentage of crude fat was when the larvae fed on the Diet 3 and it was less Percentage when the larvae fed on the Diet 4 (Berezina, 2017, Paul *et al.* 2017 , Rumpold *et al.* 2013, Schlüter *et al.* 2017).

As in many previous studies, our study showed that these larvae of *T. Molitor* are a good source of protein (Ghaly and Alkoaik 2009) and can be used to feed fish and poultry by adding them to feed, as they are highly efficient in converting protein-poor materials into a high-protein biomass (Nagasawa and Lacierda , 2004) , Shakouri and Yazdi 2012, Huis ,2013) Van *et al.* 2015 and Bureau *et al.* 2002). similar to black soldier fly, it is capable to convert waste materials to energy with higher efficiency (Abel FAS et al. 2015, Siyal et al. 2016 and Shu , *et al.* (2018).

Table (2): The growth performance of meal worms *Tenebrio molitor* throughout 30-day feeding trial.

Different Diets	LG (%)	WG (%)	SGR (%)	FCR (gm/gm)	FCE (%)
Diet (1) control	93.33±3.02 a	125.0±6.33 a	1.17±0.42 a	11.6±2.11 b	8.62±1.22 c
Diet (2)	78.57±4.11 c	114.29±2.10 b	1.10±0.11 b	10.63±3.42 c	10.39±1.45 b
Diet (3)	86.67±4.45 b	100.0±4.22 b	1.00±0.51 b	9.50±2.46 d	8.33±2.42 c
Diet (4)	26.67±2.14 e	66.67±2.46 d	0.74±0.29 c	13.15±1.62 a	7.41±1.77 d
Diet (5)	84.62±2.34 b	100.0±5.32 b	1.00±0.81 b	12.3±2.44 ab	8.13±2.93 c
Diet (6)	85.71±3.61 b	71.43±2.12 c	0.78±0.21 c	14.28±3.57 a	7.00±2.72 d
Diet (7)	80.00±2.42 c	112.5±5.41 b	1.09±0.62 b	11.82±1.92 b	8.46±1.43 c
Diet (8)	69.23±2.11 d	100.0±6.41 b	1.00±0.22 b	11.4±2.81 b	8.77±1.64 c
Diet (9)	84.61±3.71 b	125.0±7.46 a	1.17±0.32 a	11.36±1.79 b	8.80±1.33 c
Diet (10)	76.92±2.32 c	116.67±4.66 b	0.89±0.71 c	8.23±2.58 d	12.16±3.22 a
Diet (11)	66.67±1.77 d	100.00±4.93 b	1.oo±0.33 b	13.2±2.71 a	7.58±1.83 d
P value	0.00**	0.00**	0.0*	0.00**	0.00**
LSD	7.71	5.32	0.45	6.73	5.81

Means followed by same letter in column are not significantly different at 5% level.

Table 3 : Compositions of mealworms *Tenebrio molitor* larvae

Different Diets	Larval composition %				
	Crude Protein	**Moisture**	**Ash**	**Crude Fat**	**Crude Fibre**
Diet (1)	43.61 ±2.33a	8.3±0.12 b	1.9± 0.33cd	32.8±1.72 a	13.39± 1.22c
Diet (2)	44.23 ±1.43a	8.6±0.31 b	1.4± 0.12d	32.3±2.31 a	12.77±1.42 c
Diet (3)	45.72 ±1.54a	9.3±0.22 a	1.5±0.41 d	31.5±1.84 a	11.98±1.83 c
Diet (4)	36.11 ±3.66c	8.1±0.51 b	4.6± 0.88a	28.6±1.44 b	22.59±1.33 a
Diet (5)	43.52±1.78 a	9.1±0.52 a	2.2±0.37 c	31.4±1.73 a	13.18±1.56c
Diet (6)	42.13 ±1.45b	9.4±0.72 a	3.1±0.91 b	29.3±2.86 b	16.07± 1.31b
Diet (7)	40.11±1.91 b	8.2±0.61 b	2.3±0.25 c	32.7±1.56 a	16.69± 1.41b
Diet (8)	38.61±2.63 c	8.3±0.53 b	1.8±0.61 cd	30.1±1.75 b	21.19± 1.80 a
Diet (9)	43.52 ±1.51a	9.2±0.82 a	1.3±0.11 d	33.7±1.48 a	12.28± 1.77c
Diet (10)	40.45±1.33 b	9.1±0.47 a	2.1±0.22 c	31.3±1.88 a	17.05± 1.90b
Diet (11)	39.22±2.66 c	8.5±0.64 b	2.6±0.32 c	29.8±2.93 b	19.88±1.43 a
P value	0.00*	0.0*	0.00**	0.0*	0.0*
LSD	8.51	2.63	4.11	6.52	8.33

Means followed by same letter in column are not significantly different at 5% level.

Conclusion

Our study concluded that tested food materials showed good results in the survival rate of *T. Molitor* larvae and also led to improved growth and nutrition. Bran, chicken feed and oats are the best in the feeding process. The previous study suggests raising larvae on these materials when expanding on a large scale for use in food purposes for humans and animals, due to its high content of protein and fat, and therefore it is an easy and cheap source for large-scale protein production.

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تأثير اختلاف الغذاء علي معدلات النمو و تحويل الغذاء في يرقات حشرة
Tenebrio molitor L. (Coleoptera: Tenebrionidae)

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الملخص العربى

في هذه الدراسة ، تم تقييم التركيب الغذائي ومعايير نمو يرقات دودة جريش الذرة الصفراء ، *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) بعد ٣٠ من التغذية باستخدام مواد غذائية مختلفة و هي: نخالة القمح (النظام الغذائي ١) - شوفان (النظام الغذائي ٢) -علف دجاج نسبة بروتين % ٢١ (النظام الغذائي ٣) - دشيشة الذرة (النظام الغذائي ٤) - الشوفان + نخالة القمح (النظام الغذائي ٥) - الشوفان + دقيقة الذرة الذرة (النظام الغذائي ٦) - الشوفان + علف الدجاج ٢١٪ (النظام الغذائي ٧) – نخالة قمح + حاء الذرة (النظام الغذائي ٨) - نخالة قمح + علف دجاج ٢١٪ (نظام غذائي ٩) - علف دجاج ٢١٪ + دشيشة ذرة (دايت ١٠) – والنظام الغذائي الاخير عبارة عن خليط من المكونات الاربعة السابقة (نخالة قمح + دشيشة ذرة + علف دجاج ٢١٪ + شوفان (النظام الغذائي ١١) . بالنسبة لمعايير النمو فإن يرقات دودة الجريش الصفراء التي تغذت على نخالة القمح (النظام الغذائي ١) - الشوفان (النظام الغذائي ٢) - علف الدجاج ٢١٪ ومزيج منها لهما اظهرت أعلى القيم في معدل النمو وكفاءة التحويل الغذائي . أيضا أظهر التحليل الكيميائي لليرقات المرباة علي المواد الغذائية المختبرة فروقا معنوية في محتويات البروتين و الدهون و الالياف ؛ حيث أظهر التحليل أعلى نسبة من البروتين والدهون عندما تم تغذية اليرقات على النظام الغذائي ٣ و ١ ، و من ناحية أخرى ظهر أعلى مستوى من الألياف الخام عند تغذية اليرقات على دشيشة الذرة والقمح + دشيشة الذرة. بشكل عام ، و بشكل عام أظهرت النتائج أن تربية دودة الجريش الصفراء باستخدام القمح والشوفان وأعلاف الدجاج بنسبة ٢١٪ ومزيج منها كان لها تأثير إيجابي على معدل النمو والتركيب الغذائي.