Effect of insect meal as substitute for Soybean meal on performance of Ossimi lambs

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Abstract

This experiment was performed to investigate the impact of a partial substitution of 0, 10, 20, and 30% of dietary soybean meal (SBM, 43.76 % CP) by an equal portion of Oriental Hornet Meal (OHM, 76.84% CP). Forty weaned Ossimi male-lambs were assigned to four equal groups, nominated as T1 (control, 0% OHM), T2 (10% OHM), T3 (20% OHM), and T4 (30% OHM), respectively. The study lasted four months for the growth experiment, then continued until lambs reached puberty. The results showed that when lambs were fed diets containing OHM, the digestibility coefficients of dry matter, organic matter, crude protein, and nutritional values such as total digestible nutrients and digestible crude protein were considerably ($P<0.01$) higher than for control. Moreover, the final body weight and average daily gain of lambs fed OHM diets were significantly ($P<0.01$) higher compared to those fed control diet. In addition, lambs fed OHM-containing diets had a considerably greater ruminal concentration of volatile fatty acids (T2: 5.51, T3: 5.74, T4: 5.98 *vs* T1: 5.16 ml eq / 100 ml), greater counts of protozoa cells (T2: 1.33, T3: 1.45, T4: 1.67 *vs* T1: 1.22 $\times 10^6$ /ml rumen fluid) and lower concentrations of ruminal NH3-N (T2: 23.51, T3: 21.59, T4: 18.27 *vs* T1: 28.49 mg / 100 ml) versus the control diet, respectively. The levels of serum total protein, glucose, and thyroid hormones were significantly ($P<0.01$) higher by increasing OHM inclusion level, while low-density lipoprotein concentration was decreased (T2: 109.02, T3: 108.16, T4: 109.57 *vs* T1: 110.22 mg/dl). Liver and kidney functions were unaffected by groups. Moreover, the results revealed that all examined puberty parameters and the majority of semen characteristics significantly improved ($P<0.001$ and $P<0.05$) with the substitution of up to 30% of SBM with OHM, respectively. In conclusion, OHM improved the Ossimi lambs' performance, it is safe and recommended to be used.

Keywords: insect meal; sheep; growth; digestibility; reproductive response.

Introduction

Every year, the need for food derived from animals grows. This tendency corresponds to the rising human inhabitants and a greater public understanding of the value of consuming animal protein. By 2050, the United Nations anticipates that there will be 9.5 billion people on the planet (Henchion et al., 2017; Kim et al., 2019; Hopkins et al., 2021). At the same time, the Food and Agricultural Organization (FAO) stated that in request to meet the demand of the world's population, animal food production should be increased by 60–70% (Makkar et al., 2014). It is common knowledge that feed costs make up between 60 and 70 percent of the total cost of animal farms (Kim et al., 2019). Especially since the cost of feeds high in protein is expected to rise further (Chia et al., 2019). The lack of resources for producing protein feed and its high cost are the main issues facing the world's animal farming industry. Soybean meal is one example of a conventional feed material with very high prices and limited future availability. Traditional protein ingredients that are added to feed, such as soybean or cottonseed meal, place additional strain on nonrenewable resources (Sogari et al., 2019). As the human population grows, lack and expensive traditional protein feed resources induced high-pressures nutritionists to find cheap alternative feed protein sources that reduce feeding costs and the environmental impact on meat production, likewise edible insects.

Edible insects as a novel protein substitute will undoubtedly increase soon. Edible insects became interesting dietary sources of protein due to their greater amount of protein than conventional protein sources such as meat, dairy products, and nuts. However, insect protein is regarded as a complete protein comparable to that of milk and cow proteins, and its digestibility is similar to that of chicken eggs (Shockley and Dossey, 2013). Edible insects are insects that can be consumed by both humans and domesticated animals. For centuries, humans in many countries have consumed insects (Henchion et al., 2017; Kim et al., 2019; Magara et al., 2021; Pasini et al., 2022). From these perspectives, it is promising to use edible insects' high-quality protein as livestock feed rather than human food (Kim et al., 2019). Most insects contain an optimal ratio of the essential amino acids, for instance, methionine and lysine (Makkar et al., 2014; Al-Qazzaz and Ismail, 2016). The FAO highly suggests adding insect protein to the diets of poultry in order to enhance growth and production measurements as well as reduce feed costs (FAO, 2014). However, the majority of research on feeding insects to farm animals has focused on chickens (Józefiak et al., 2016), aquaculture (Belghit et al., 2018), and ducks (Gariglio et al., 2019). In recent studies, the use of edible insects as a replacement for expensive soybean and fish meal in farm animal feeds has been highlighted (Sogari et al., 2019).

Ruminants are the livestock species that have been the least studied in terms of consuming edible insects. Just a few studies, for instance, using insect meal as a further source of protein for beef steers (Fukuda et al., 2022), goats (Astuti et al., 2019), in vitro rumen fermentation (Jayanegara et al., 2017a; Jayanegara et al. 2017b; Jayanegara et al. 2020). Ahmed et al. (2021) concluded that there was no negative impact on nutrient digestibility from replacing 25% of soybean meal in the ruminant rations with the four tested insects.

There are many species of edible insects out their BSFL (*Hermetia illucens*), mealworms (*Tenebrio molitor*), and house flies (*Musca domestica*) (Sogari et al., 2019), *Vespa Orientalis L* (El-Sheikh et al., 2022). which has an advantageous effect on animal health, performance, digestibility, and the quality of the final product. *Vespa Orientalis L*, also referred to as the Oriental Hornet, is an extremely dangerous insect that preys on honeybee colonies all over the world and poses a significant issue for beekeepers and losses in agriculture (Abou-Shaara, 2017; El-Sheikh et al., 2022). Therefore, gathering these *Vespa* species may be a method of biocontrol, but it may also be used as animal feed (Ghosh et al., 2021). Oriental Hornet are easily caught with traps and bait. Wasp traps are regarded as affordable because they can be produced using local raw materials (El-Sheikh et al., 2022). Furthermore, these catches can accumulate huge numbers of wasps as well as lowering the price per unit of protein production.

Applying OHM in farm animal feeds has received little research like Józefiak et al. (2016), most recently, El-Sheikh et al. (2022) and Mohamed et al. (2023). Therefore, it's crucial to determine the nutritional value of Oriental Hornet (Van Huis et al., 2013). Despite the paucity of research on using insects to feed Ossimi lambs, this study investigated the effects of feeding OHM to Ossimi lambs on performance, nutrient digestibility, and some blood parameters.

Materials and Methods

This study was conducted at Fac. of Agri. Exp. Station, Minia Univ., with latitude 28.1229° N and longitude 30.7347° E in partnership with the Sids Experimental Station in the Beni-Suef Governorate,

Animal Production Research Institute (APRI), Agricultural Research Centre (ARC), Ministry of Agriculture, Egypt,

Oriental Hornet harvesting and preparation

In two places near Baraka Apiaries, Kfer Shibin in Shibin Al Qanater, Qalyubia Governorate, Egypt. Fifty triangular traps were employed to obtain oriental wasps. In the wasp traps, there was attractive bait that didn't contain any chemicals. The attractive bait cake, which was placed in the trap's drawer, had Sugar, sugar fermented syrup, honeybee, and supplement Baraka organic. The trap has three funnels and is made from locally sourced materials (wire gauze, wooden bars, and a stainless steel cone with Moshtohor feeder and baits). About 25 kg of wasp were used during the experimental period at a total cost of approximately 240 L E as a substitute for 36 kg of soybean meal, which cost approximately 288 L E.

Animals, rations, and management

This study used forty male Ossimi lambs starting post-weaning with an initial body weight of 20.58 ± 0.85 kg. The growth experiment lasted 120 days then continued until lambs reached puberty. Based on their initial body weight, animals were assigned at random into four equal groups (ten each) to be fed on four experimental rations to study the effect of using insect meal (OHM), the cheap protein source as a substitute for soybean meal. The control ration (Con) consisted of 60% concentrate feed mixture (CFM) and +40% rice straw (RS) to cover their nutrients requirements according to NRC (1985). The CFM in the control ration consists of 20% soybean meal, 33% yellow corn, 42% wheat bran, 3% molasses, 0.5% sodium chloride. 1% limestone and 0.5% vitamins and mineral mixture (Table, 1). Treatments were nominated as T1 (Control, 0% OHM), T2 (10% OHM), T3 (20% OHM) and T4 (30% OHM), where OHM was incorporated on the account of soybean meal as a replacer of its proportion in the control treatment (Table 1). At 8 a.m. and 4 p.m., rations were distributed in two equal portions. Water was always available; feed remains were gathered and weighed every day and daily feed intakes were also noted. At the beginning of the experiment and every two weeks after that, the lambs' body weights were measured and calculated average daily gain (ADG). The amount of dry matter (DM), TDN, and DCP needed to generate a one-kg weight gain was calculated as the feed conversion.

Digestibility and chemical analysis

At the end of the study period, digestion evaluations were conducted using metabolic cages to determine the nutritional digestibility, feeding values, and rumen parameters of the tested rations. Five animals from each group were fed individually for a total of 15 days of trial, 10 days of adaptation, and 5 days of data collection, followed by three days of rumen studies.

Rations, Soybean meal, Oriental Hornet Meal (OHM) and faeces samples were analyzed for dry matter, organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash according to (AOAC, 1985). Nitrogen free extract (NFE) was calculated by the difference. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to (Van Soest et al., 1991). Hemicellulose was calculated by difference (NDF – ADF). Table 1 showed the chemical composition of soybean meal (SBM), Oriental Hornet meal (OHM), and experimental rations.

Ruminal liquor parameters

After the digestibility trials, stomach tubes were used to collect rumen liquor samples from 20 lambs (5 each), at 0 times (before feeding), 3 hours after feeding, and 6 hours after feeding. Three layers of surgical gauze were used to filter the samples. Then immediately, A digital pH meter was used to determine the ruminal pH, rumen ammonia-N was measured in accordance with (Conway, 1957), and total volatile fatty acids (TVFA's) were measured by the steam distillation method as described by (Warner, 1964). The total number of protozoa was counted by using the Fuchs Rosenthal chamber. According to Schultz and Schultz (Shultz and Shultz, 1970), the sodium tungstate method determined microbial protein.

Blood samples

The collection of blood samples was performed every two weeks from each animal by extracting them through the jugular vein into sterile tubes prior to the lamb's morning feeding and continued until the completion of the trial. To obtain the serum, the collected blood samples underwent centrifugation at a speed of 4500 revolutions per minute (rpm) for a duration of 20 minutes. A colorimetric technique described by Cannon (1974) was used to measure serum total protein and albumin and the difference

between total proteins and albumin was used to calculate serum globulin. According to Howanitz et al. (1984), serum glucose was measured calorimetrically. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured in accordance with Young (2000) instructions. To analyze the concentrations of urea-N and cholesterol in the blood serum, a range of methods and kits were employed. For the urea-N concentration, a spectrophotometer was utilized, along with Spinreact, S.A.U kits obtained from Ctra. Santa Coloma, 7 E-17176 Sant Esteve de Bas (GI), Spain. Cholesterol concentration, the same spectrophotometer was utilized in conjunction with Spinreact, S.A.U kits from the aforementioned source. Shifting the focus to testosterone concentration, the method developed by Jaffe and Behrman (1974) was employed. This method assessed total testosterone concentration using Coat Acount 125I radioimmunoassay (RIA) kits purchased from Diagnostic Products Corporation in Los Angeles, California, 90045, USA.

Sexual activity and seminal characteristics

Male lambs were examined for libido and sexual activity every day between 8:00 and 9:00 a.m. until puberty. Two estrus ewes were placed in separate pens, one ewe per pen. These pens were then separated by a third similar pen, which allowed for observation by the observer. The ewes were left unrestrained in their respective pens. To evaluate the male lambs, a unique method was employed. Each male lamb was randomly selected and evaluated individually. They were exposed to the two ewes for a duration of 20 minutes on each test day. This approach aimed to assess the lamb's behavior and preferences towards the ewes. The remaining lambs, not participating in the evaluation process, were placed approximately 20 yards away from the test pens. Visual barriers were set up to ensure the separation between the test lambs and the observers. This separation aimed to prevent any potential interference or influence on the evaluation process. Throughout the experiment, each pen was utilized for the test lambs from all groups. This allowed for a fair and consistent evaluation process across the different male lambs and groups involved. The experiment focused on capturing several key factors related to sexual behavior. Scrotal circumference (SC) was measured as an indicator of testicular development. This measurement involved using a flexible tape to wrap around the widest point of the testes, capturing the maximum circumference of the paired testes. Reaction time (RT) was another crucial aspect measured during the experiment. It involved recording the duration, in minutes, from the time the ewe was introduced to the ram until the ram began to mount and ejaculate. This measurement aimed to gauge the ram's level of interest, arousal, and readiness for sexual activity. Lastly, the latency period (LP) was measured to assess the duration, in minutes, after ejaculation until the ram resumed sexual activity. This measurement aimed to understand the ram's recovery period and its readiness for subsequent sexual encounters. By incorporating these comprehensive measurements, the experiment sought to gain insights into the sexual behavior and preferences of the male lambs towards the estrus ewes. The measurements for reproduction were conducted in accordance with Kridli and Al-Yacoub (2006). Puberty was the age at which the first ejaculate containing motile sperm was collected. Lambs were trained for semen collection after the first mounting with erection. Male lambs were stimulated sexually by permitting two false mounts, particularly prior to the first ejaculation, followed by two minutes of restraint, another false mounting, two more minutes of restraint, and finally an ejaculation (Almquist and Hall, 1973). An artificial vagina was used to collect the semen. During the process of collection, the temperature within the inner liner rubber of the artificial vagina was carefully regulated to a range of 41-43°C. To ensure utmost hygiene, a fresh inner liner and a graduated collecting tube were employed for each collection. Before use, the inner liner was lubricated with sterile Vaseline to facilitate the process. Promptly following each collection, the semen from the ejaculate was swiftly transported to the laboratory. Here, the volume of each ejaculate was meticulously measured in milliliters, with utmost precision down to the nearest 0.1 ml. This measurement was carried out using a transparent graduated plastic tube. Two successful ejaculates are evaluated separately for spermatozoa characteristics using the methodology outlined by Salisbury (1978). A drop of sperm was used to test the motility of sperm using a microscope at low power (×10) and a heated slide set to 37°C. A hemocytometer was used to calculate the number of spermatozoa per milliliter of semen and to estimate the percentage (%) score for progressive motility. Mathematical calculations were made for the sperm index and total motile sperm output/ ejaculate.

Economical features

The tested rations' economic evaluation was calculated. One kilogram of live weight gain in sheep costs 50 LE, and one ton of concentrate feed mixture costs 4.809, 4.807, 4.804, and 4.802 LE, respectively, for T1, T2, T3, and T4. Rice straw cost 1100 LE/Ton. The following equations were used to calculate the return:

Total revenue/head (LE) = Total body weight gain × the price for each Kg gain was LE (50).
Net revenue /lamb (LE) = Total revenue / h -Total feed cost/h.
Economic efficiency = Net revenue /h (LE)/ Total feed cost/ h (LE)

Statistical analysis

The results were presented as the mean ± SE of the mean. In order to identify significant differences between all tested treatments, statistical analyses were performed using the General Linear Model (GLM) of SAS (SAS, 2020), and Duncan's New Multiple.

Results

Chemical compositions

Chemical compositions of SBM, OHM, and experimental rations are listed in Table 1. The CP contents of SBM and OHM used in the current study were 43.76 and 76.84 %, respectively. The content of NDF observed in OHM was much greater as compared to SBM, the values were (25.26%) and (13.15%), respectively. While the ADF content of the insect meal (9.55%) is near to that of SBM (10.25%). Results of EE contents between SBM and OHM were almost similar, the values were (2.67%) and (2.84%), respectively. The four diets that were tested have a chemical compatibility range of 12.69 to 12.93% for crude protein, and the gross energy content (4.08 to 4.09 Mcal/Kg DM) is within the suggested range for the optimal performance and growth of sheep (Council, 1985). The average of NDF ranged from 40.74 to 41.39%, while the average of ADF ranged from 23.08 to 23.11%.

Digestibility coefficient

Data of Table (2) showed the visible digestibility of DM, OM, CF, CP, NDF, NFE, and ADF in Ossimi lambs fed the experimental rations. The values of DM and OM digestibility were significantly ($P< 0.01$) increased for Ossimi rams fed (OHM20 and OHM30) rations compared with those fed (Con and OHM10) rations. Additionally, the digestibility of crude protein (CP) was significantly (P 0.01) higher in Ossimi rams fed diets containing insect meal OHM than those fed the control diet. Ossimi rams fed the control diet (Con) had considerably lower ether extract (EE) digestibility than rams fed (OHM30) ration. No significant difference was found in the digestibility of crude fiber (CF), NDF and ADF among treatments. It's interesting to note that the nutritional value of the Oriental Hornet Meal (OHM) groups especially, (OHM20 and OHM30) were superior to the control treatment in terms of TDN and DCP values ($P<0.01$).

Rumen fermentation

Table (3) displays the findings for ruminal pH values, volatile fatty acid (VFA's) levels, and ammonia nitrogen (NH3-N) concentrations. Data on ruminal pH showed that values were not significantly changed at zero time and slightly changed at 3, 6 hrs. with no significant differences between treatments. The highest ruminal pH readings for all dietary treatments were obtained prior to feeding, while the lowest readings were found three and six hours after feeding. Also, the overall average of ruminal pH was not changed by treatments. The concentrations of VFA's and ruminal NH3-N were lowest at zero time and tended to rise thereafter being the highest at 3 hrs. post feeding for ruminal NH3-N and at 6 hrs. post feeding for ruminal VFA's concentrations. According to the overall average data, the control group had the highest ruminal NH3-N concentrations, while the least values were obtained with the insect meal groups (OHM10, OHM20 and OHM30). However, the highest ruminal VFA's concentrations were recorded with treated groups, while the least values were obtained with the control group.

Performance and Production Responses

The following table (4) displays the values of growth performance, feed intake, nutrient intake, feed conversion and economic efficiency of growing lambs fed diets including OHM as a protein source substitution for SBM. With an increase in the supplementation amount of OHM, the FBW, ADG, and growth rate (GR) all enhanced in a highly significant ($P<0.01$). The TDMI/kg gain, TDN/kg gain, and DCP/kg gain all showed similar trends in feed conversion. Also, feeding Oriental Hornet Meal (OHM) in place of soybean meal (SBM) in growing lamb rations increased the total feeding cost by 11.36, 16.14 and 18.68 % compared to those fed control ration, respectively. However, the total revenue was increased by 17.1, 26.83 and 30.83% upon feeding rations T2, T3 and T4 compared with T1, respectively. Thus, substituting OHM meal for soybean meal in lambs diets at the studied percentages (10, 20 and 30%) increased the economic advantage by 9.57, 16.52 and 19.13% respectively, compared to the control group.

Table 1. Chemical composition of treatment rations

Items	SBM	OHM	Experimental rations			
			T1 Con	T2 OHM10	T3 OHM20	T4 OHM30
Rice straw (RS)	---	---	40	40	40	40
Concentrate feed mixture (CFM); consisted as follows:	---	---	60	60	60	60
Oriental Hornet MEAL (OHM)			0	1.14	2.28	3.42
Soybean meal (SBM)	---	---	20	18	16	14
Yellow corn	---	---	33	33	33	33
Wheat bran	---	---	42.00	42.86	43.72	44.58
Molasses	---	---	3	3	3	3
Sodium chloride	---	---	0.5	0.5	0.5	0.5
Limestone	---	---	1	1	1	1
Vitamins and mineral mixture	---	---	0.5	0.5	0.5	0.5
Total			100	100	100	100
nutrients analysis:						
Dry matter %	88.51	96.76	85.17	85.23	85.29	85.35
GE (Mcal/Kg DM)[1]	4.70	5.25	4.08	4.08	4.08	4.09
Crude protein %	43.76	76.84	12.69	12.77	12.85	12.93
Ether extract %	2.67	2.84	1.73	1.73	1.72	1.71
Crude fiber %	7.64	0.44	15.15	15.11	15.08	15.04
NDF%	13.15	25.26	40.74	40.95	41.17	41.39
ADF%	10.25	9.55	23.08	23.09	23.10	23.11
Hemicellulose % [2]	2.90	15.71	17.65	17.86	18.07	18.28
Nitrogen-free extract %	39.08	14.05	61.18	61.16	61.14	61.11
Ash %	6.85	5.83	9.25	9.24	9.22	9.21

1 GE calculated after Nehring and Haenlein (Nehring and Haenlein 1973). GE, Mcal/kg DM = (5.72 CP +9.5 EE +4.79 CF + 4.03 NFE) / 1000 (where the values of CP, EE, CF, NFE, are in g/kg DM). 2 Hemicellulose = %NDF - % ADF

Table 2. Digestibility coefficient and nutrient values of Ossimi lambs fed the experimental rations

Items	Experimental rations				SEM	P. value
	T1 Con	T2 OHM10	T3 OHM20	T4 OHM30		
Digestibility coefficient, %						
DM	70.98^{c}	71.32^{c}	72.34^{b}	73.30^{a}	0.273	0.0013
OM	61.89^{b}	62.77^{b}	64.03^{a}	64.88^{a}	0.367	0.0019
CP	69.67^{d}	72.77^{c}	76.63^{b}	79.41^{a}	0.382	<.0001
EE	63.31^{b}	63.67^{b}	64.10^{ab}	65.24^{a}	0.387	0.0357
CF	63.59	63.42	64.01	65.34	0.484	0.0837
NDF	51.43	50.32	50.71	51.58	0.328	0.0942
ADF	46.80	46.16	46.69	46.92	0.431	0.0737
NFE	69.97	70.21	70.64	70.68	0.247	0.1990
Nutritive values, %						
TDN	63.75^{c}	63.99^{c}	64.54^{b}	65.31^{a}	0.154	<.0004
DCP	8.84^{d}	9.29^{c}	9.84^{b}	10.27^{a}	0.048	<.0001

$^{a, b, c \text{ and } d}$ Values within a row with different superscripts differ significantly at $P < 0.05$

Table 3. Rumen activity of Ossimi lambs fed experimental rations

Rumen liquid parameters	Time of samples	Experimental rations				SEM	P. value
		T1	T2	T3	T4		
		Con	OHM10	OHM20	OHM30		
pH	Zero time	7.19	7.20	7.18	7.21	0.014	0.5957
	After 3hr	6.53	6.55	6.50	6.49	0.044	0.7273
	After 6hr	6.30	6.29	6.25	6.22	0.043	0.5675
	Overall mean	6.67	6.68	6.64	6.64	0.021	0.4121
NH3-N (mg / 100 ml)	Zero time	16.72^{a}	13.86^{b}	12.10^{c}	10.73^{d}	0.352	<.0001
	After 3hr	36.44^{a}	30.23b	27.99^{b}	23.44^{c}	1.015	0.0001
	After 6hr	32.30^{a}	26.45^{b}	24.69^{b}	20.64^{c}	0.976	0.0002
	Overall mean	28.49^{a}	23.51^{b}	21.59^{c}	18.27^{d}	0.484	<.0001
Total volatile fatty acids (ml eq / 100 ml)	Zero time	4.69^{b}	5.07^{a}	5.14^{a}	5.23^{a}	0.094	0.0172
	After 3hr	5.13^{c}	5.48^{b}	5.81^{a}	5.88^{a}	0.085	0.0009
	After 6hr	5.66^{d}	5.99^{c}	6.27^{b}	6.82^{a}	0.073	<.0001
	Overall mean	5.16^{d}	5.51^{c}	5.74^{b}	5.98^{a}	0.048	<.0001
Total number of protozoa (10^{6} /ml rumen fluid)	Zero time	0.60^{b}	0.78^{a}	0.81^{a}	0.84^{a}	0.032	0.0029
	After 3hr	1.34^{c}	1.41^{c}	1.59^{b}	1.94^{a}	0.034	<.0001
	After 6hr	1.73^{c}	1.81b^{c}	1.96^{b}	2.22^{a}	0.050	0.0006
	Overall mean	1.22^{d}	1.33^{c}	1.45^{b}	1.67^{a}	0.023	<.0001
Microbial protein (g/100ml rumen fluid)	After 6hr	0.52^{c}	0.60^{b}	0.63^{a}	0.65^{a}	0.01	<.0001

$^{a, b, c \text{ and } d}$ Values within a row with different superscripts differ significantly at P < 0.05

Table 4. Growth performance, feed efficiency, and economic efficiency of Ossimi lambs fed experimental rations.

Items	Experimental rations				SEM	P. value
	T1	T2	T3	T4		
	Con	OHM10	OHM20	OHM30		
Growth performance						
Initial weight, kg	20.67	20.50	20.50	20.67	0.48	0.9889
Final weight, kg	41.17^{c}	44.50^{b}	46.50^{ab}	47.50^{a}	0.74	<.0001
Total body gain, kg	20.50^{c}	24.00^{b}	26.00^{a}	26.83^{a}	0.61	<.0001
Avg. body gain, g/day[3]	170.83^{c}	200.00^{b}	216.67^{a}	223.61^{a}	5.06	<.0001
Growth rate (%)	99.49^{b}	117.65^{a}	127.03^{a}	130.18^{a}	4.24	0.0002
Feed intake						
CFM / h / d, kg	0.71^{c}	0.80^{b}	0.84^{a}	0.85^{a}	0.011	<.0001
RS / h / d, kg	0.51^{b}	0.54^{a}	0.55^{a}	0.57^{a}	0.011	0.0038
TDMI / h / d, kg[1]	1.22^{c}	1.34^{b}	1.39^{ab}	1.42^{a}	0.021	<.0001
TDN / h / d, kg	0.91^{c}	1.00^{b}	1.05^{a}	1.09^{a}	0.016	<.0001
DCP / h / d, kg	0.11^{d}	0.12^{c}	0.14^{b}	0.15^{a}	0.003	<.0001
Feed conversion (FC)						
FC (TDMI/ kg gain)	7.13^{a}	6.71^{b}	6.42^{b}	6.35^{b}	0.122	0.0008
FC (TDN/ kg gain)	5.31^{a}	5.02^{b}	4.87^{b}	4.87^{b}	0.091	0.0090
FC (DCP/ kg gain)	0.63	0.62	0.63	0.65	0.012	0.3837
Economic efficiency						
Price / kg CFM (LE)*	4.809	4.807	4.804	4.802	-	-
Price / kg RS (LE)*	1.1	1.1	1.1	1.1	-	-
Total Feed cost / h / 120d, LE	477.35^{c}	531.57^{b}	554.40^{a}	566.52^{a}	7.61	<.0001
Total revenue / h, LE[2]	1025.00^{c}	1200.00^{b}	1300.00^{a}	1341.67^{a}	30.39	<.0001
Net return / h, LE[3]	547.65^{c}	668.43^{b}	745.60^{a}	775.14^{a}	25.58	<.0001
Economic efficiency[4]	1.15^{b}	1.26^{ab}	1.34^{a}	1.37^{a}	0.041	0.004
Relative EE%	100.00	109.60	117.22	119.26	-	-

* Based on prices in the Egyptian market during the experimental period. [1]TDMI= Total dry matter intake
[2] Total revenue/lamp (LE) = Total body gain × 50, assuming that the selling price of each Kg gain was LE (50).
[3]Net revenue /lamp (LE) = Total revenue / h -Total feed cost/h. [4]Economic efficiency = Net revenue /h (LE)/ Total feed cost/ h (LE). $^{a, b, c \text{ and } d}$ Values within a row with different superscripts differ significantly at $P < 0.05$

Table 5. Blood biochemical parameters of Ossimi lambs fed experimental rations

Items	Experimental rations				SEM	P. value
	T1	T2	T3	T4		
	Con	OHM10	OHM20	OHM30		
Biochemical parameters						
Total protein (g/dl)	6.16^{d}	6.45^{c}	6.76^{b}	7.18^{a}	0.070	<.0001
Albumin (g/dl)	4.00^{c}	4.18^{bc}	4.36^{b}	4.63^{a}	0.072	0.0015
Globulin (g/dl)	2.16	2.27	2.40	2.54	0.131	0.2703
A/G ratio	1.87	1.85	1.84	1.83	0.134	0.9966
Glucose (mg/dl)	69.01^{b}	73.64^{a}	74.68^{a}	77.11^{a}	1.35	0.0168
Lipid profile						
Total cholesterol (mg/dl)	171.78^{b}	168.86^{b}	172.50^{ab}	176.97^{a}	1.51	0.0316
Triglyceride (mg/dl)	61.11	57.33	55.00	55.33	1.58	0.0865
HDL (mg/dl)	49.33^{b}	48.37^{b}	53.33^{a}	56.33^{a}	0.98	0.0015
LDL (mg/dl)	110.22	109.02	108.16	109.57	1.84	0.8763
VLDL (mg/dl)	12.22	11.47	11.00	11.07	0.32	0.0866
Liver function						
AST (U/L)	39.36	39.26	39.11	38.74	0.553	0.8637
ALT (U/L)	26.92	26.85	26.16	26.21	0.834	0.8672
Kidney function						
Urea (mg/dl)	42.12	43.38	43.98	44.07	0.97	0.4994
Creatinine (mg/dl)	0.96	0.95	0.84	0.88	0.049	0.3601
Thyroid hormones						
T_3 (nmol/L)	1.55^{d}	2.14^{c}	2.30^{b}	2.44^{a}	0.382	<.0001
T_4 (nmol/L)	29.98^{b}	32.72^{b}	34.27^{ab}	35.68^{a}	0.387	0.0357

$^{a, b, c \text{ and } d}$ Values within a row with different superscripts differ significantly at $P < 0.05$

Table 6. Puberty parameters of Ossimi rams fed experimental rations

Items	Experimental rations				SEM	P. value
	T1 Con	T2 OHM10	T3 OHM20	T4 OHM30		
Age (day)	300.33^{a}	275.33^{b}	257.67^{c}	239.67^{d}	5.00	0.0002
Body weight (kg)	37.33^{d}	40.67^{c}	42.67^{b}	44.67^{a}	0.53	<.0001
Testes circumference (cm)	19.67^{d}	22.00^{c}	23.67^{b}	25.67^{a}	0.50	0.0002
Urethral process (cm)	2.13^{c}	2.53^{b}	2.67^{b}	2.97^{a}	0.04	<.0001
Reaction time (min)	10.53^{a}	8.39^{b}	6.14^{c}	5.33^{c}	0.34	<.0001
Latency period (min)	15.66^{a}	13.64^{b}	11.29^{c}	9.20^{d}	0.55	0.0002
Testosterone level (ng/dl)	196.20^{c}	222.99^{b}	237.61^{b}	252.83^{a}	4.64	0.0002

$^{a, b, c \text{ and } d}$ Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 7. Semen properties of pubertal rams fed experimental rations

Semen characteristics	Experimental rations				SEM	P. value
	T1 Con	T2 OHM10	T3 OHM20	T4 OHM30		
Seminal volume (ml)	0.97^{b}	1.06^{ab}	1.17^{a}	1.23^{a}	0.05	0.0341
Progressive motility (%)	65.00^{b}	71.67^{ab}	71.67^{ab}	76.67^{a}	2.04	0.0240
Sperm conc. /ml ($\times 10^{9}$)	1.05^{d}	1.26^{c}	1.41^{b}	1.60^{a}	0.04	<.0001
Sperm output/ej. ($\times 10^{9}$)	1.02^{d}	1.34^{c}	1.65^{b}	1.97^{a}	0.07	<.0001
Motile sperm/ml ($\times 10^{9}$)	0.69^{c}	0.90^{b}	1.01^{b}	1.23^{a}	0.05	0.0006
Motile sperm/ej. ($\times 10^{9}$)	0.45^{c}	0.65^{b}	0.73^{b}	0.94^{a}	0.06	0.0017
Live sperm (%)	73.33	78.33	78.33	80.00	2.88	0.4411
Abnormality (%)	25.33^{a}	20.33^{b}	17.00^{b}	12.33^{c}	1.35	0.0009
Semen index[1]	4853.85^{c}	7577.60^{b}	9236.50^{b}	12113.10^{a}	718.6	0.0007

[1]Semen index = Seminal volume (ml) × Progressive motility (%) × Sperm conc. /ml ($\times 10^{9}$) × Live sperm (%)

$^{a, b, c \text{ and } d}$ Values within a row with different superscripts differ significantly at $P < 0.05$.

Physiological response

Table, (5) displays the impact of OHM-supplemented meals on the blood biochemical parameters, lipid profile, Liver functions, kidney functions and Thyroid hormones content of growing lambs. When compared to the T1 group, the serum total protein, albumin, and glucose levels in the T2, T3, and T4 groups were all significantly higher ($P< 0.01$ and $P< 0.05$, respectively). The lipid profiles showed that Total cholesterol and HDL levels were considerably greater in the T4 group than in the T1 group (P 0.05), although there was no significant difference ($P > 0.05$) in the levels of Triglyceride, LDL, or VLDL between the groups. Liver enzymes were not affected by treatments. Also, at the same line kidney function (creatinine and urea concentrations) is not affected by OHM diets. However, Thyroid hormones affected by OHM supplementation to Ossimi lambs ration result in significantly ($P< 0.01$) increased T3 level (ng/ml) compared to those fed control ration. And tended to increase T4 levels for OHM10 and OHM20, with a significant ($P<0.05$) increase for OHM30 compared with the control.

Reproductive Responses

Puberty parameters and semen properties of pubertal Ossimi male-lambs fed experimental ratios are shown in Tables (6 and 7). The results revealed that the replacement of SBM protein up to 30 % with OHM protein improved significantly ($P < 0.001$ and $P < 0.05$) in all studied puberty parameters and most semen characteristics, respectively. The results indicated that the treated lambs significantly ($P < 0.001$) reached puberty at a younger age and heavier body weight than the untreated lambs. Furthermore, testicular circumference, reaction time, and latency period all increased considerably with puberty and continued to do so until sexual maturity. Moreover, the T4 group was the best one and had a significant ($P < 0.001$) higher value in testosterone levels followed by T3 and T2 groups compared to the T1 group the lowest one. The percentages of enhancement in lambs fed OHM protein (T2, T3, and T4) were 13.65%, 21.11% and 28.86%, respectively, in testosterone levels compared to the control fed SBM protein (T1).

Discussion

Chemical compositions

Insects are one possible protein source for livestock feed. High CP contents of OHM as an insect meal in this experiment were in line with El-Sheikh et al. (2022) and some other studies reported that the CP content of insect meal from around 13 and 77 % (Tao and Li, 2018) and 15 to 81 %, (Ramos-Elorduy et al., 1997) others, 39% up to 64.4% (Moyo and Moyo, 2022). Ahmed et al. (2021)demonstrated that the four different insect species' chemical compositions had higher protein percentages, ranging from 52.4 to 61.3% than SBM 48.3%. This considerable variation in N concentration in insect meals could be attributed to changes in development stage, the rearing process (particularly feed composition), and N determination technique (Rumpold and Schlüter, 2013; Sánchez-Muros et al., 2014). The Oriental Hornet meal (OHM) had NDF contents 25.26% While SBM had roughly half the NDF content of (OHM), Whereas the ADF content of the insect meal (9.55%) is near to that of SBM (10.25%), this may explain the difference in hemicellulose content of SBM and OHM. Rea (2022) evaluated 14 edible insects as feed for cattle and illustrated that there was a variation in NDF and ADF among samples, both within and between species, the values ranged from 14.19 to 55.43% for NDF and from 9.05 to 40.36% for ADF. At the same time, Renna et al. (2022) reported that insect meal had a higher NDF concentration ranging from 22.5 to 28.2%, while soybean meal had 11.3% NDF. Furthermore, they stated that ADF content ranged from 5.8 to 9.3%, whereas SBM content was 6.3%. In ruminant nutrition, both conventional and alternative protein sources had minimal fat content (Halmemies-Beauchet-Filleau et al., 2018). The examined Oriental Hornet meal had low levels of EE (2.84%); Thus, it renders it an alternative to conventional protein sources such as SBM 2.67%, which is similar to the composition of EE (El-Sheikh et al., 2022). Rea (2022) found that the lowest EE content was (grasshopper meal) with 4.67%, and 3.94% for Silkworm pupae meal (SWP). On the other hand, Rashmi et al. (2022) claimed that insect meal had more EE than SBM.

Digestibility coefficient

There hasn't been much research that investigates how substituting insect meal impacts ruminants digestibility. The improvement in digestibility of DM, OM, CP, EE, and Nutrient values TDN and DCP for rations containing insect meal especially on OHM 30 ration could be due to the chitin content in Oriental Hornet meal (OHM). El-Sheikh et al. (2022) declared that the chitin contents for *VESPA ORIENTALIS* meal was 34.53% on a DM basis. Chitin and chitosan supplementation enhanced bovine production performance, digestibility, ruminal activity, and bacterial community structure, according to a number of studies (Del Valle et al., 2017; Pereira et al., 2018; Zanferari et al., 2018). They reported

that adding chitosan to lamb feed improved feed intake, digestibility's of NDF, DM, CP, nitrogen balance, and microbial protein production. Chitosan is found in the exoskeleton of insects (Li et al., 2018). Earlier research has assessed the use of insects or insects oil at various levels in ruminant meals and show that the insects had a lower nutritional value because they cause a reduction in the DM and OM digestibility by reason of high-fat content and chitin, which may inhibit rumen microbes (Jayanegara et al., 2017a; Jayanegara et al., 2017b; Jayanegara et al., 2020). In contrast to earlier results, the inclusion various level of insects employed as SBM substitutes in the current study showed no negative impacts on nutritional digestibility, which could be attributable to the low EE composition of OHM and the low levels of OHM (10, 20, and 30%) replacement of the dietary soybean meal that represents 20% of the concentrate mixture which make up 60% of the whole diet.

Our results of digestibility coefficient agreed with Phesatcha et al. (2022) conducted the impacts of various levels of cricket meal (CM) as apart of soybean meal (SBM). They discovered that replacing SBM with CM increased DM degradability significantly (p 0.05). Also, RAMOS et al. (1981) assessed the digestibility of multiple edible insect species. They found that the digestibility of organic matter varied between species from 77.9% to 98.9% and the digestibility of proteins, which make up more than 60% of most species, varied between 45% and 66.9%. Moreover, Narang and Lal (1985) discovered that the substitution of *Alphitobius diaperinus* for soybean meal protein significantly increased nitrogen digestibility. Furthermore, Ahmed et al. (2021) concluded that adding the four tested insects to the ruminant diet in place of 25% of soybean meal had no negative effects on the nutrient digestibility. On other species, Gariglio et al. (2019) demonstrated that the digestibility of EE was higher in the insects meal (black army fly larva) treatments of ducks against to the control treatment. On the other hand, Fukuda et al. (2022) estimated the impacts of inclusion Black Soldier Fly larvae (*Hermetia illucens*) BSFL on beef steers diets as a protein supplement. And they discovered that the digestibility of DM, OM, or NDF was not significantly affected by treatments.

Rumen fermentation

The reduction in rumen pH values at 3- and 6-hours post feeding can be attributed to the increase in concentrations of rumen VFA's at those times. The ruminal pH-decreasing potential of volatile fatty acid accumulation was shown by the inverse relationship between VFA's levels and pH in the rumen (Dijkstra et al., 2012). However, the reduced concentrations of ammonia in the rumen of rams fed insects meal than those fed the control ration can be attributed to increased incorporation of ammonia into microbial protein or it's absorption across the rumen wall and this was clear in our study by an increase of microbial protein and total number of protozoa with rations containing insect meal. That was supported by Jayanegara et al. (2017b) they suggested that the number of proteolytic bacteria and protozoa was significantly ($p<0.05$) enhanced with substituted soybean meal by 15% cricket meal than control ration containing 30% soybean meal. Additionally, Bach et al. (2005) illustrated that NH3 can be used to synthesis of microbial proteins, and it can be absorbed into the bloodstream through the rumen wall. Also, Castillo-Lopez and Domínguez-Ordóñez (2019) concluded that bacteria and protozoa were the main producers of microbial protein. Furthermore, total VFA's are the results of their activity and OM degradation as well as carbons utilization for microbial protein synthesis (Dijkstra et al., 1992). This is clear from the results of improved OM digestibility, and increased VFA's concentrations by the inclusion of OHM in the ration.

The results were in agreement with Fukuda et al. (2022) who studied the impact of BSFL as a protein source for steers diets. They discovered that no effect of treatment on ruminal pH tended to lower ammonia. Also, Jayanegara et al. (2017b) supplementation of BSF resulted in lower ammonia concentration. In addition, Renna et al. (2022) illustrated that the production of ammonia was lowest in most insect meals than SBM. Furthermore, Phesatcha et al. (2022) found that increasing levels of cricket meal (CM) replacing SBM had a significant improve in total VFA especially in the 75:25 ratio for SBM:CM with a value of 78.5 compared with 59.8 mmol/L for the control diet. On the other hand, Ahmed et al. (2021)stated that the inclusion of different insect meals in diets had a significant increase in pH compared with diets containing SBM. Intriguingly, adding insect meals to the diet made NH3-N levels rise. Renna et al. (2022) reported that the final rumen pH of insects meals was significantly higher than that of plant meals (on average 7.0 vs. 6.7, respectively). Astuti et al. (2019) illustrated that applying cricket meal in place of up to 30% of the soybean meal in the concentrate had no adverse effects on the rumen fermentation profiles. The rumen fermentation activity was supported by all parameters being in normal ranges.

Performance and Production Responses

The inclusion of OHM in their diet may have resulted in a significant enhancement in the growth performance of Ossimi lambs in the present investigation This was consistent with Fukuda et al.'s

(Fukuda et al. 2022) discovery that beef steers fed insect meal diets increased their intake, encouraging weight gain. In addition, Astuti et al. (2019). Reported that in comparison to the control, Kids goats fed a milk substitute that contained insect meal (IM) consumed more dry matter, protein, and fat intake. Also, they reported that Pre-weaning goats and sheep could have an ADG of between 100 and 120 g/h/d if BSF and IM were included in milk replacer, while post-weaning goats and sheep could have an ADG of more than 150 g/h/d if those ingredients were included in creep feed. Furthermore, they informed that goats fed meals that includes 30% cricket meal as a substitute for soybean meal had final weights of 23.19 kg, ADG of 136.54 g/h/d and Feed efficiency of 23.24%. Which were only slightly improved than those of the control diets of 21.79 kg, 135.30 g/h/d and 20.44%, but the differences were insignificant.

The enhancement of growth performance may be explained by increasing feed intake and the rise in the paternal CP digestibility, DCPI, and nutritive values (DCP and TDN), which had an impact on the performance of their lambs. In this regard, some reports support the results that have been provided by demonstrating the beneficial effects of supplementary insect meals on animal performance. Which were concur with the favorable response for the inclusion of insect protein in lambs diets may be due to the high feed intake (Oibiokpa et al., 2018), insect meal has a high biological value protein content that supports a sufficient supply of essential amino acids (Khan et al., 2016), and biologically active substances found in the insect protein, such as anti-inflammatory peptides, insulin regulators, and antioxidant agents (Acosta-Estrada et al., 2021). Our study's findings are consistent with the findings reported by Adeniji (2007), who discovered that diets with meat and bone meal cost much more than diets with insect meal. Recent research by Renna et al. (2022) revealed that silkworm pupae meal (SWP) could provide one unit of crude protein for only half the cost of soybean meal. On other species, Hatab et al. (2020) found that the diets were more economically beneficial versus the control diet, when Japanese quail were fed 100% insect meal instead of animal protein

Physiological Response

The impact of insect meal on the blood constituents in ruminants has only been briefly discussed in a few studies. The variations in an animal's metabolic profile are mainly controlled by nutrition (Coroian et al., 2017). Notably, the increase in TP concentration in the OHM groups was related to an enhancement of the digestibility of CP and nutritive values as DCP in the same groups in comparison with the control group. It is the point of interest to see the increase in blood glucose when the dietary OHM was raised from 10 to 30%. These results ascertain the improvement obtained in TDMI. No significant differences were detected in liver enzymes (AST & ALT) or kidney function (urea & Creatinine) concentrations among the studied treatments. This may give a signal that the digestibility and metabolizable processes were working normally without stress and the dietary ingredients were free from any infectious agent. Renna et al. (2022) the normal range of blood urea nitrogen, Creatinine, and liver enzymes (AST & ALT) and indicates that SWP supplementation had no effect on liver integrity or function.

Considering the thyroid gland activities through its secretions, synthesis, and storage of thyroxin (T_4) and triiodothyronine (T_3) hormones, again ration that contain OHM showed considerable improvement in the concentrations of both hormones. This result is compatible with the enhancement attained in FBW and BWG as these hormones target the metabolic processes in the whole body (Husveth, 2011). Hart et al. (1981) reported the relationship between thyroid hormones secretion and the metabolism of such nutrients.

Reproductive Responses

A wide range in lambs' growth rates and elevated serum testosterone levels may be the cause of the observed differences in puberty ages and weights in the current study. Improvement in the treated groups (T2, T3, and T4) in parameters of sexual desire and puberty can be attributed to increased feed intake, digestibility, and BWG. It is confirmed by studies of Mekoya et al. (2009) and Freitas et al. (2004). A strong correlation between testosterone levels and age at sexual development, according to Vijayakumar et al. (2019). Additionally, starting spermatogenesis at puberty and continuing it until maturity requires testosterone (Castro et al., 2002). These findings could help clarify why reaction time significantly decreased in lambs fed OHM as opposed to those fed a control diet. On the other hand, Wells et al. (2003) reported that thyroxine might be an indicator of the beginning of puberty because thyroxine levels steadily increase from low levels just before the beginning of the breeding season to peak levels just before an estrus transition.

All studied semen properties such as volume, motility, total sperm output, motile sperm output, live & abnormal spermatozoa, and semen index of Ossimi lambs fed OHM protein in diets recorded im-

provement compared to lambs fed SBM protein (control). The semen index had 56.12, 90.29, and 149.56 % enhancement in the T2, T3, and T4 groups, respectively compared to the T1 group. These results agree with those reported by Hernandez et al. (2011) and Mohamed et al. (2016) who suggested that nutrition plays a significant role in initiating puberty in lambs and early reaching sexual maturity, mating age, and production system that are all closely linked to the feeding regimen. The positive synergistic impact of feeding on OHM protein could be responsible for the influences observed for the treated groups in this research that promoted nutrient digestibility and rumen activity, thus growth and reproductive performance. *Vespa Orientalis* meal-supplemented ration boosted V-line bucks' daily sperm output, testicular sperm reserves, FSH, LH, testosterone hormone, and their ability to antioxidants (Mohamed et al., 2023). It has been revealed that animals' bodily weight, age, and sperm reserves are highly correlated (Osinowo et al., 1981). The enhancement of semen characteristics may be attributed to elevating body weight, testes circumference, and testosterone levels (Mohamed et al., 2016). The improvement of reproductive organs and their activities are significantly influenced by testosterone. El-Kholy et al. (2008) showed that the amount of testosterone, which controls the activity of spermatogenesis and accessory sex glands, was responsible for the improved semen characteristics.

Conclusion

According to our results, we can conclude that Oriental Hornet Meal (OHM) meal could be used as a protein source to partially replace soybean meal, up to 30%, to improve productive, reproductive performance, nutrient values, physiological responses, and economic efficiency without any negative effects on Ossimi lambs' performance.

Ethics approval

All studies were approved by Animal and Poultry Production Department, Faculty of Agriculture, Minia University, Egypt Following the recommendations of the Ethics Committee for the care and use of animals, microorganisms, and living cell cultures in education and scientific research. Ethics Number: MU/FA/014/12/22.

Data and model availability statement

The data that support the findings of this study are available on request from the corresponding author. Software and data repository resources

None of the data was deposited in an official repository.

Declaration of interest

None.

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