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The effects of the dietary replacement of soybean meal with yellow mealworm larvae (Tenebrio molitor) on the growth, nutrient digestibility and nitrogen output of fattening rabbits

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ABSTRACT

The present study aimed to obtain novel information concerning the effects of the total dietary replacement of soybean meal (SBM) with yellow mealworm (Tenebrio molitor; TM) on the coefficients of total tract apparent digestibility (CTTAD) in the diet and on the nitrogen output of fattening rabbits. Two diets were formulated that included 60 g/kg SBM (SBM diet) or 30 g/kg TM (TM diet) as the main crude protein (CP) source. The TM diet had slightly lower CP content than the SBM diet (141 vs. 150 g/kg). A total of 30 rabbits (Hyplus PS 19 x Hyplus PS 40; males) weaned at the age of 32 day were randomly allocated into 2 groups (15 rabbits per group), individually housed, and fed one of the two diets ad libitum for 47 days. Feed intake was measured daily during the entire fattening period (between 32 and 79 day of age). The total faecal and urine output were individually measured between 57 and 61 day of age. A lower daily weight gain (ADWG) between 32 and 53 day of age (by 6.9 g/day, 12 %; P = 0.034), resulting in lower live weight (LW) at the age of 53 day, and a lower feed intake (ADFI) between 32 and 79 day of age (by 13 g/day, 7 %; P = 0.047) were recorded in rabbits fed the TM diet compared to rabbits fed the SBM diet. The ADWG or feed conversion ratio concerning the entire fattening period, the final LW of rabbits, or the CTTAD of the diets were not affected by treatments. Whatever the diet, on average, losses of nitrogen in faeces represented 26 % of the nitrogen intake. Lower losses of nitrogen in urine (by 0.35 g/day, 32 %; P = 0.007) and total excretion of nitrogen (by 0.53 g/day, 23 %; P = 0.003), as well as a higher nitrogen retention coefficient (by 0.06, 11 %; P = 0.026) were detected in rabbits fed the TM diet compared to rabbits fed the SBM diet. It can be concluded that fattening rabbits effectively utilized the diet containing TM without detrimental effects on the CTTAD of the diets or on their nitrogen output.

Keywords: Rabbit, Soybean meal, Insect meal, Nutrient digestibility, Nitrogen output

1. Introduction

With the estimated increasing global population and a limited amount of arable land, there is undoubtedly a need to tackle problems related to satisfying the demand for animal products (Van Huis and Tomberlin, 2017; Van Huis and Oonincx, 2017). In this respect, feed is the most challenging issue because of food-feed competition (e.g. soy products, diverse cereals), fishmeal referring to ocean overfishing, ongoing climate change, land degradation and water shortages (Makkar et al., 2014; Dicke, 2018; Makkar, 2018). If this problem is to be solved, identifying human-inedible resources is necessary. Currently, algae, leaf proteins, seaweeds, coproducts of the biofuel industry, protein isolates, slaughterhouse and food waste, as well as insect meal, are considered to have the potential to replace human-edible components in the diets of food-producing animals (Van Krimpen and Hendriks, 2019; Makkar, 2018).

There is increased interest of nutritionists in using dietary insect meal as a feed for food-producing animals (Van Huis and Oonincx, 2017; Van Huis, 2020). In general, insects have attracted the attention of nutritionists because of their ability to convert agricultural by-products or other organic waste materials into high-quality food or feed. The use of insects as food or feed may represent the efficient use of land and water sources (Van Huis and Oonincx, 2017; De Souza-Vilela et al., 2019; Pinotti et al., 2019), and concurs with several of the Sustainable Development Goals of the United Nations concerning sustainably nourishing the growing human population (Dicke, 2018).

From an animal nutrition point of view, the larvae of the black soldier fly (Hermetia illucens), housefly (Musca domestica), silkworm (Bombyx mori) and yellow mealworm (Tenebrio molitor; TM) are considered suitable feed for poultry, pigs, fish and shrimp (Van Huis and Tomberlin, 2017; Dicke, 2018; Makkar, 2018). In general, insects have a high protein content that is highly digestible, and so are the lipid contents (Makkar et al., 2014). The chemical composition of insect species can be affected by the species, life stage, sex and diet (Finke and Oonincx, 2017; Kulma et al., 2019). In the case of diet, however, it seems that amino acid (AA) and minerals contents tend to be less variable (Finke and Oonincx, 2017). Regarding consumer acceptance, Sogari et al. (2019) suggested that there is no barrier towards the consumption of animals fed insects.

Information on the dietary inclusion of insects as a protein source in rabbit feed is very scarce (Carregal and Takahashi, 1987; Gugofek et al., 2019). It seems, however, that rabbits are able to effectively utilize animal products as a dietary source of protein (Lebas, 2004). For our study, we used TM, as it is one of the above mentioned the insects suitable for livestock feed. We hypothesized that TM might be an effective substitute for soybean meal (SBM) in rabbit diets during the fattening period. To our knowledge, no previously conducted research has investigated the dietary inclusion of TM in rabbit diets.

Therefore, the aim of this study was to evaluate the effect of the total dietary replacement of SBM by TM on the coefficients of total tract apparent digestibility (CTTAD) of the diets, the nitrogen output and the growth of fattening rabbits.

2. Materials and methods

The protection of animals used in this study respected the rules stated in EC Directive 86/609/EE (EEC, 1986), and the study was carried out upon approval of the Ethics Committee of the Institute of Animal Science (the code of the approval: E5/2018, Prague, Czech Republic

2.1. *Experimental diets*

The chemical composition of the TM (Tenebrio molitor) (Papek s.r.o., Popovice u Jaromenc nad Rokytnou, Czech Republic) and SBM used in this study is presented in Table 1. Crushed vegetables, peeled potatoes and fruit were used as feed for TM, whereas bran and feeding meal were used as a bedding straw.

Table 1 Chemical composition of soybean meal (SBM) and Tenebrio molitor larvae meal (TM) (g/ kg on an as-fed basis) used in this study.

	SBM	TM
Determined values		
Dry matter	891	594
Crude protein	465	400
Ether extract	24	150
aNDFom[a]	117	115
ADFom[b]	70	83
Ash	63	34
Ca	2.7	0.7
P	6.2	7.6
Mg	2.8	2.5
K	19	11.1
Na	0.2	1.35
Lysine	27.8	18.6
Methionine + cysteine	12.6	8.2
Threonine	16.9	13.0
Arginine	32.2	15.1

[a]Neutral detergent fibre assayed with a heat stable amylase and expressed excluding residual ash.

[b]Acid detergent fibre content is expressed excluding residual ash.

The primary components of TM were crude protein (CP) and ether extract (EE), whereas ash, neutral detergent fibre (aNDFom) and acid detergent fibre (ADFom) were present in smaller amounts. Yellow mealworm larvae used in this study had a low Ca content and a low Ca to P ratio, whereas the K content was high. Two diets were formulated and included 60 g/kg SBM (SBM diet) or 30 g/kg TM (TM diet) as the main protein source (Table 2). The diets had a similar basal mixture of ingredients. The TM diet contained more wheat bran (by 20 g/kg on an as-fed basis) and barley (by 10 g/kg on an as-fed basis) than the SBM diet. The diets included no additional fats or synthetic AA. The experimental diets had similar levels of EE, aNDFom, ADFom and AA, and were designed to have a similar digestible protein/digestible energy ratio (Table 2). The TM diet had slightly lower CP and lysine contents than the SBM diet. The diets were offered to all rabbits as 3-mm pellets with a length of 5—10 mm. No a coccidiostatics additive or antibiotics were included in the diets used in this study.

2.2. *Rabbit husbandry and experimental design*

The study was carried out in an experimental rabbit room equipped with air conditioning. The animals were kept under the following controlled environmental conditions: room temperature 16 °C, relative humidity 65 % and 12-h/d light programme.

A total of 30 Hyplus rabbits (PS 19 x PS 40; males; 32 day of age) were used to assess the CTTAD of organic matter (OM), CP (nitrogen x 6.25), aNDFom, ADFom and gross energy (GE) of the experimental diets, using the methodology of Perez et al. (1995), and to determine the nitrogen balance and nitrogen retention. Rabbits were also used to determine growth performance. Rabbits were split into 2 groups (15 rabbits per group) and were provided with one of the two diets (SBM or TM diet) ad libitum for 47 days. Rabbits were individually housed in wire net metabolic cages (50 x 40 x 42.5 cm). The live weight (LW) of the animals was recorded at weekly intervals. The feed intake was measured daily during the entire fattening period (between 32 and 79 day of age). Similarly, the health status of the animals was checked daily. The total faecal and urine output (between 57 and 61 day of age) were measured as described by Volek et al. (2020), and samples were stored at —18 °C for analysis. Data obtained during the experiment were used for calculation of the average daily weight gain (ADWG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

Table 2 Ingredients and chemical composition (g/kg on an as-fed basis unless otherwise stated) of the experimental rabbit diets based on soybean meal (SBM diet) or yellow mealworm (Tenebrio molitor, TM diet).

	Diets	
Item	SBM	TM
Ingredient		
Alfalfa meal	300	300
Soybean meal	60	–
Yellow mealworm	–	30
Wheat bran	330	350
Sugar beet pulp	70	70
Oats	150	150
Barley	60	70
Vitamin–mineral supplement[a]	10	10
Dicalcium phosphate	5	5
Limestone	10	10
Salt	5	5
Analysed composition (n = 2)		
Dry matter	898	880
Crude protein	150	141
aNDFom[b]	350	351
ADFom[c]	183	180
Lignin (sa)[d]	50	50
Ether extract	25	26
Lysine	6.7	6.2
Methionine + cysteine	5.2	5.1
Threonine	5.8	5.5
Gross energy (MJ/kg)	16.2	16.1
Ash	77	71
Nutritive value[e]		
Digestible protein	111	105
Digestible energy (MJ/kg)	9.3	9.3
Digestible protein/digestible energy ratio (g/MJ)	11.9	11.3

[a] *Provides the following quantities per kilogram of complete diet: vitamin A (retinol), 12,000 IU; vitamin D3 (cholecalciferol), 2000 IU; vitamin E (a-tocopherol), 50 mg; vitamin K3 (bisulphite menadione complex), 2 mg; vitamin B1 (thiamine mononitrate), 3 mg; vitamin B2 (riboflavin), 7 mg; vitamin B6 (pyridoxine), 4 mg; niacinamide, 50 mg; Ca-pantothenate, 20 mg; folic acid, 1.7 mg; biotin, 0.2 mg; vitamin B12 (cyanocobalamin), 0.02 mg; choline chloride, 600 mg; Cu (as CuSO4*5H2O), 15 mg; Fe (as FeSO4*7H2O), 50 mg; I (as KI), 1.2 mg; Mn (as MnO), 47 mg; Zn (as ZnO), 50 mg; Se (as Na2SeO3).*

[b] *Neutral detergent fibre assayed with a heat stable amylase and expressed excluding residual ash.*

[c] *Acid detergent fibre is expressed excluding residual ash.*

[d] *Lignin determined by solubilisation of cellulose with sulphuric acid.*

[e] *Nutritive values of the experimental diets were calculated from digestibility coefficients (Table 3).*

2.3. Analytical methods

All chemical analyses (SBM, TM, diets, faeces, urine) were performed in duplicate. The procedures of AOAC International (2005) were used to determine the contents of dry matter (method 934.01), ash (method 942.05), CP (method 954.01) and EE (method 920.39). The nitrogen levels in the SBM, TM, diets, faeces and urine were determined using a Kjeltec Auto 1030 Analyser (FOSS Tecator AB, Hoganas, Sweden). The EE levels in the feeds and the SBM and TM were determined using Soxtec 1043 (FOSS Tecator AB, Hoganas, Sweden). Neutral detergent fibre was determined using a methodology of Mertens (2002), whereas procedure 973.18 of AOAC International (2000) was followed for ADFom determination. Lignin levels were determined as described by Robertson and Van Soest (1981). The GE was measured using an adiabatic calorimeter (C5000 control, IKA-Werke, Staufen, Germany). The AA contents in the diets and SBM and TM used in this study were determined as previously described by Volek et al. (2020). Briefly, the samples of the diets and SBM and TM were hydrolyzed in 6 M hydrochloric acid at 110 °C for 23 h and analyzed using an AA Analyzer AAA-400 (INGOS Ltd., Prague, Czech Republic). The Ca, P, Mg, K and Na contents in the TM were determined by atomic absorption spectrometry performed using a Solaar M6 instrument (TJA Solutions, Cambridge, UK).

2.4. Statistical analyses

One-way analysis of variance using the GLM procedure in SAS (Statistical Analysis Systems Institute, 2006) was used for analysis of experimental data obtained in this study, taking into account the type of diet as the fixed effect. The experimental unit concerning CTTAD, nitrogen balance, nitrogen retention and growth performance represented the individual rabbits. Differences between the least square means were considered significant at $P < 0.05$.

3. Results

3.1. Growth, nutrient digestibility and nitrogen output in rabbits

No mortality or morbidity was observed during the entire fattening period.

The growth performance, CTTAD of the diets and nitrogen balance and retention are presented in Table 3. A lower ADWG in the first 3 weeks after weaning (by 6.9 g/day, 12 %; $P = 0.034$), resulting in lower LW of rabbits at day 53 (by 150 g, 8 %; $P = 0.055$), and a lower ADFI during the entire fattening period (by 13 g/day, 7 %; $P = 0.047$) were detected in rabbits fed the TM diet compared to tho fed the SBM diet. No differences were observed between treatments in terms of the ADWG or FCR during the entire fattening period, the final LW of rabbits at 79 day.

The diets were not significantly different with respect to the CTTAD of the OM, CP, aNDFom, ADFom or GE.

Table 3 Growth performance during the fattening period (32 to 79 day of age), coefficients of total tract apparent digestibility (CTTAD), nitrogen balance and nitrogen retention in rabbits fed diets based on soybean meal (SBM diet) or yellow mealworm (Tenebrio molitor, TM diet).

	Diet		RMSE[a]	P-value
	SBM	TM		
Live weight (g)				
at 32 day of age[b]	742	736	60	0.798
at 53 day of age	1962	1812	190	0.055
at 79 day of age	3213	3089	246	0.211
Average daily weight gain (g)				
32–53 day of age	58.1	51.2	7.8	0.034
32–79 day of age	52.2	49.9	4.6	0.216
Average daily feed intake (g)				
32–53 day of age	135	123	18	0.091
32–79 day of age	180	167	16	0.047
Feed conversion ratio				
32–53 day of age	2.37	2.40	0.22	0.665
32–79 day of age	3.46	3.35	0.18	0.102
CTTAD[c]				
organic matter	0.579	0.586	0.025	0.514
crude protein	0.738	0.743	0.029	0.660
aNDFom	0.259	0.267	0.035	0.564
ADFom	0.124	0.123	0.051	0.959
gross energy	0.571	0.577	0.026	0.561
Nitrogen balance[c] (g/day)				
N intake	4.78	4.15	0.52	0.014
N excretion in faeces	1.26	1.07	0.24	0.092
N excretion in urine	1.08	0.73	0.25	0.007
Total N excretion	2.34	1.81	0.35	0.003
N retention				
retained N[d] (g/day)	2.44	2.33	0.37	0.501
coefficient of N retention[e]	0.51	0.57	0.05	0.026

[a] *RMSE = root mean square error (n = 15 males per diet; individually housed in wire-net cages).*

[b] *At weaning.*

[c] *Determined in rabbits between 57 and 61 days old.*

[d] *As nitrogen intake-total nitrogen excretion (faeces + urine).*

[e] *As retained nitrogen/nitrogen intake.*

The nitrogen intake was lower in the rabbits fed the TM diet (by 0.63 g/day, 13 %; P = 0.014) than in the rabbits fed the SBM di< The losses of nitrogen in faeces were not significantly affected by the dietary treatment. Lower losses of nitrogen in urine (by 0.35 ; day, 32 %; P = 0.007) were detected in rabbits fed the TM diet than in rabbits fed the SBM diet. Consequently, the total excretion nitrogen was lower in rabbits fed the TM diet (by 0.53 g/day, 23 %; P = 0.003). The nitrogen retention coefficient was higher in rabb: fed the TM diet (by 0.06, 11 %; P = 0.026) than in rabbits fed the SBM diet.

4. Discussion

4.1. Chemical composition of the yellow mealworm (Tenebrio molitor) used in this study

The values reported here for the protein and lysine, sulphur AA, threonine and arginine contents, as well as for the EE, aNDFom a ADFom contents, are similar to those reported by others (Finke, 2002; Makkar et al., 2014; Finke and Oonincx, 2017). The low < content and low Ca to P ratio observed in the present study are typical for insects (Makkar et al., 2014; Makkar, 2018; Finke, 2002). , found in other studies (Finke, 2002; Makkar et al., 2014), TM contained more K than Na. Regarding fibre fractions determined in tl present study, and in insects generally, sometimes, ADFom is used for the estimation of the chitin content (Finke and Oonincx, 2017). should be taken into account, however, that this method overestimates chitin levels (Finke, 2007; Finke and Oonincx, 2017).

4.2. Rabbit growth performance

In the present study, a lower growth rate in the first 3 weeks after weaning and a lower feed consumption during the entire fatteni period were recorded in rabbits fed the TM diet compared to those in rabbits fed the SBM diet. Regarding the whole fattening perio however, ADWG, FCR and the final LW of rabbits were not affected by dietary TM inclusion. These results are probably associated wi the lower CP content in the TM diet. In fact, Maertens et al. (1997) recorded a significantly lower growth rate and feed intake with tl reduction of the dietary protein supply during the first 3 weeks post-weaning, whereas during the finishing period, the rabbits gain more weight from the reduced protein supply. The lower feed consumption of rabbits fed the TM diet might be also associated with lower acceptance of dietary TM inclusion. To our best knowledge, however, there is no relevant information concerning the pal ability of diets containing insect meal in rabbits in the literature. The results of the growth performance of rabbits recorded in t present study should be confirmed using a larger sample size.

4.3. Crude protein digestibility, nitrogen balance and nitrogen retention in rabbits

The main CP sources used in this study (SBM vs. TM) did not affect the CTTAD of CP. Regardless of the dietary treatment, < average, losses of nitrogen in faeces accounted for 26 % of the nitrogen intake, which is in accordance with Calvet et al. (2008). In tl present study, lower losses of nitrogen in urine (40 % of the total excretion of nitrogen) were observed in rabbits fed the TM diet than rabbits fed the SBM diet (46 % of the total excretion of nitrogen). Usually, urine and faeces contribute to the total excretion of nitrogi in approximately the same proportion (Calvet et al., 2008; Gidenne et al., 2013; Volek et al., 2020). In this respect, the lower losses nitrogen in urine observed in the present study might be explained by the moderate protein content in the TM diet (141 g/kg). In fai Gidenne et al. (2017) observed that urine contributed to only 20 % of nitrogen excretion in rabbits fed a diet with a CP level of 1 g/kg. Consequently, in our study, the total excretion of nitrogen was lower in rabbits fed the TM diet, and although the nitrogen intal was also lower in rabbits fed the TM diet, the nitrogen retention coefficient was significantly higher in rabbits fed this diet. This findi is likely due to the lower CP content in the TM diet compared to that in the SBM diet. Maertens et al. (1997) reported that nitrogi retention in rabbit increases with decreasing dietary protein content. On the other hand, a possible different AA/CP balance of die used in this study might be also responsible of the differences observed in urine nitrogen output between diets.

5. Conclusion

It can be concluded that fattening rabbits effectively utilized a diet containing 30 g yellow mealworm (Tenebrio molitor)/kg. In tl present study, a total dietary replacement of SBM with yellow mealworm (Tenebrio molitor) had no detrimental effect on the digesti of nutrients or the nitrogen output in fattening rabbits.

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