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Article

The effect of silkworm pupae and mealworm larvae meals as dietary protein components on performance indicators in rabbits

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Abstract:

This study aimed to evaluate the effect of feeding rabbits with silkworm pupae and mealworm larvae meals on their performance indicators. Ninety (90) rabbits were divided into three groups. Control group (C) was fed with 10% soybean meal (SBM), SPM group received the diet including 5 % SBM and 4 % of silkworm pupae meal, and MLM group received the diet including 5 % SBM and 4 % of mealworm larvae meal. The body weight of rabbits and average daily gains were determined. Feed conversion ratio (FCR) was calculated. At the end of fattening period, the animals were euthanized, skinned and eviscerated to determine their carcasses characteristic. Hind leg and loin muscles were collected for analyses of the chemical composition. At the end of fattening period, rabbits from groups SPM and MLM were heavier than C rabbits (2,606.5 and 2,584.8 *vs* 2,404.0 g), which also improved their overall carcass characteristic while FCR was similar between groups. However, feeding rabbits with the addition of insect's meals increased the amount of ether extract in their muscles. Based on the results obtained, it may be concluded that SBM may be partially replaced by silkworm pupae and mealworm larvae meals in rabbit diets.

Key words: Growth performance, Mealworm larvae meal, Rabbit feeding, Silkworm pupae meal, Soybean meal substitution.

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Introduction

The use of different species of insects as a source of dietary protein and fat has been increasingly addressed recently. In many countries, invertebrates are a popular source of protein in compound feeds for livestock. Farm animals are fed with larvae of the black soldier fly (*Hermetia illucens*)[1], the house fly (*Musca domestica*)[2], mealworm (*Tenebrio molitor*)[3,4], silkworm (*Bombyx mori*)[5] as well as insects of the order *Orthoptera*, i.e. locusts and crickets[6].

In many European countries, the main source of protein for broiler rabbits is imported extracted soybean meal and, to a lesser extent, rapeseed meal and sunflower meal[7,8]. Other protein sources are also tested, such as legume seeds and DDGS[9,10,11]. Literature on the use of insects in rabbit feeding is scarce, although interest in this subject has increased in recent years. As the first, the possibility of replacing soybean meal with silkworm chrysalis meal was studied[12]. Recently, the possibility of adding *Tenebrio molitor* oil and *Hermetia illucens* fat to rabbit diets was explored[13,14,15]. The feeding of insect meals to herbivorous animals is currently prohibited in Europe to minimize the risk of transmission of transmissible spongiform encephalopathies (TSEs). In addition, insect meals are expensive in Europe, therefore their use in animal nutrition is economically unjustified. It is worth noting, however, that Liu *et al*[16] consider silkworm pupae not as a treatment factor but as a normal ingredient of rabbit diets, which may be indicative of their widespread use in China. The Derwent Innovations Index database of Web of Science (accessed 31 Dec 2018), lists Chinese patents for feeding rabbits with yellow mealworm powder (four patents) and silkworm pupae 23 patents) in addition to other dietary ingredients.

It can be hypothesized that feeding growing rabbits with insect meals may be a viable alternative to soybean meal feeding. The objective of this study is to highlight the effect of feeding rabbits with silkworm pupae meal and mealworm larvae meal on their performance indicators.

Material and methods

The study was carried out in Aleksandrowice located in southern Poland, Europe (50º04'53" N and 19º45'48" E). The project was approved by the local ethics committee (case no. 1192/2015).

The experimental factor was the contribution of silkworm pupae meal and mealworm larvae meal in pelleted feed mixtures. The chemical composition and energy value of these components and of the soybean meal (SBM) have been determined (Table 1). The control feed mixture (C group) contained 10 % extracted soybean meal (SBM). In the first experimental group (SPM), the diet contained 5 % SBM and 4 % of silkworm pupae meal. In the second group (MLM), the diet included 5 % soybean meal and 4 % of mealworm larvae meal (Table 2). Chemical composition and energy content of pelleted feed mixtures have been determined (Table 3).

Table 1: Chemical composition (% of DM) and measured energy content (MJ/kg) of soybean meal, silkworm pupae meal and mealworm larvae meal

	Soybean meal	**Silkworm pupae meal**	**Mealworm larvae meal**
Dry matter	893.5	944.0	943.0
Crude ash	67.3	44.0	34.0
Crude protein	502.6	517.5	513.4
Ether extract	21.5	241.9	279.5
Neutral detergent fibre	150.2	64.9	114.2
Acid detergent fibre	78.4	54.9	75.9
Acid detergent lignin	39.6	24.6	12.6
Lysine	32.7	29.0	28.2
Methionine + cystine	14.1	21.3	10.7
Threonine	19.1	21.1	21.6
Tryptophan	6.6	7.1	6.1
Gross energy	16.38	23.94	22.50

Table 2: Ingredients of feed mixtures (%)

	Group		
	Control	**SPM**	**MLM**
Soybean meal	10	5	5
Silkworm pupae meal	0	4	0
Mealworm larvae meal	0	0	4
Alfalfa meal	25	25	25
Rapeseed meal	4	4	4
Corn DDGS[1]	4	4	4
Wheat bran	25	26	26
Ground wheat	5	5	5
Ground barley	10	10	10
Dried beet pulp	7	7	7
Arbocel[2]	5	5	5
Fodder yeast	1	1	1
Dried whey	1	1	1
Salt	0.2	0.2	0.2
Ground limestone	1.3	1.3	1.3
Feed phosphate	0.5	0.5	0.5
Vitamin-mineral premix[3]	1	1	1
Total	100	100	100

[1] Dried distillers' grains with solubles.

[2] Crude fibre concentrate.

[3] 1 kg: vit A 3 500 000 IU, vit D_3 200 000 IU, vit E 28 000 mg, vit K_3 200 mg, vit B_1 1 500 mg, vit B_2– 2 800 mg, vit B_6 2 800 mg, vit B_{12}– 20 000 mcg, folic acid 200 mg, niacin 10 000 mg, biotin 200 000 mcg, calcium pantothenate 7 000 mg, choline 30 000 mg, Fe 17 000 mg, Zn 2 000 mg, Mn 1 000 mg, Cu (copper sulfate x $5H_2O$, 24.5%) 800 mg, Co 1 000 mg, I 100 mg, methionine 150 g, Ca 150 g, P 100 g.

Table 3: Chemical composition (% of DM) and measured energy content (MJ/kg) of feed mixtures

	Group		
	Control	**SPM**	**MLM**
Dry matter	892.0	894.0	894.0
Crude ash	79.3	78.3	78.0
Crude protein	187.1	187.7	187.6
Ether extract	31.2	40.1	41.4
Neutral detergent fibre	274.6	271.3	273.0
Acid detergent fibre	154.1	153.0	153.8
Acid detergent lignin	36.1	35.5	35.0
Lysine	8.7	8.6	8.5
Methionine + cystine	5.2	5.5	5.1
Threonine	6.9	7.0	7.1
Tryptophan	2.4	2.4	2.4
Gross energy	16.86	16.95	16.94

Ninety New Zealand White (NZW) rabbits were divided into three equal groups, being analogous in terms of origin, proportion of sexes, and body weight. The experiment was carried out from September to October and started when rabbits were weaned at 35 d of age and terminated when they reached 90 d of age. Rabbits were kept in a closed experimental pavilion, in wire net flat- deck cages (0.5 × 0.6 × 0.4 m; 1 animal each), and were fed pelleted diets *ad libitum*. They were kept under standard conditions: temperature of 18 to 20 °C and relative air humidity of 60 to 75 %, intensive ventilation of rooms, and regulated photoperiod (16-h lighting and 8-h darkness).

The rabbits were weighed on an electronic scale on d 35, 56, 70 and 90 (n= 30). These data allowed calculating daily body weight gains (DBWG) of the rabbits and the feed conversion ratio (FCR): body weight gains (g)/feed intake (g). At the end of the production trial, after 24-h fasting, the animals were weighed and killed according to the accepted recommendations for euthanasia of experimental animals (rabbits were stunned and bled, and the whole procedure took about 2 min.). After the slaughter, the animals were skinned and eviscerated. After cooling the carcasses (for 24 h, at 4 ºC), muscle samples (hind leg and loin, n= 20) were taken for chemical analyses, and dressing percentage (DP; n= 20) was calculated as follows: DP (%) = chilled carcass weight without head and giblets (kg) / live weight (kg) × 100%.

Chemical composition of feed and animal muscles was determined by AOAC[17] standard methods in duplicate samples. Dry matter content was determined in a laboratory drier, at 103 ºC. Crude ash content was estimated by sample mineralization in a muffle furnace (Czylok, Poland) at 600 ºC. Total nitrogen content was determined by the Kjeldahl method, in the FOSS TECATOR Kjeltec 2200 Auto Distillation Unit. Ether extract content was estimated by the Soxhlet method, in the FOSS SOXTEC SYSTEM 2043. NDF (neutral detergent fibre), ADF (acid detergent fibre) and ADL (acid detergent lignin) were estimated in the FOSS TECATOR Fibertec 2010 System. NDF was determined according to the procedure proposed by Van Soest *et al*[18]. ADF and ADL were determined according to procedures of AOAC[17]. The levels of amino acids were determined using the Biochrom 20 plus amino acid analyser and Biochrom amino acid analysis reagents (Biochrom Ltd., Cambridge, England). Gross energy content was determined using a bomb calorimeter (IKA® C2000 basic, Germany).

Data are expressed as means ± standard error of the mean (SEM). The results were processed statistically using least squares means in GLM procedures. For comparison of data, the $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + \varepsilon_{ijk}$ model was used, where μ is the general mean, α_i is the effect of diet, β_j is the effect of sex, $\alpha_i\beta_j$ is the interaction effect between diet and sex, and ε_{ijk} is the random error. The significance of the differences among groups was determined with Duncan's multiple range test. Analyses did not reveal significant effects of sex or significant interactions between fixed effects, therefore they are not reported in the tables. Calculations were made with Statistica software[19].

Results

Body weights of rabbits at 35 d were similar between groups, however, when measured at 56 d, rabbits from MLM group were heavier than rabbits from the control and SPM groups (Table 4). At 70 d, differences in body weight were noted between all three groups. The highest body weight was observed in MLM rabbits (1,954.1 g), followed by SPM (1,883.3 g) and C rabbits (1,818.3 g). At the end of fattening period, rabbits from groups SPM and MLM were heavier than control rabbits (2,606.5 and 2,584.8 *vs* 2,404.0 g). Accordingly to the body weights measured, DBWG from 35 to 56 d was higher in MLM group (28.93 g/d) than in C group (22.38 g/d) and SPM group (23.31 g/d). No significant differences were observed between the groups in daily weight gains of the rabbits from 57 to 70 d. In the final fattening period, DBWG of the SPM rabbits were higher than those calculated for the other groups (36.16 g/d in SPM vs. 29.28 g/d in C and 31.53 g/d in MLM). In general, DBWG of the rabbits from 35 to 90 d were higher in SPM (33.2 g/d) and MLM groups (32.9 g/d) than in C group (only 29.6 g/d). FCR was similar between groups and ranged from 3.62 g/g in SPM group to 3.65 g/g in C group. We did not record any losses of animals during growth period.

Table 4: Growth performance, daily body weight gains, feed conversion ratio and mortality of rabbits (mean±SEM)

	Group			***P***
	Control	**SPM**	**MLM**	
BW 35 d, g	778.3±9.6	782.5±6.6	773.3±7.2	0.717
BW 56 d, g	1,248.3±34.2^{b}	1,272.1±22.9^{b}	1,380.8±24.7^{a}	0.004
BW 70 d, g	1,818.3±35.9^{c}	1,883.3±10.3^{b}	1,954.1±35.3^{a}	0.010
BW 90 d, g	2,404.0±27.2^{b}	2,606.5±23.1^{a}	2,584.8±21.5^{a}	<0.001
DBWG 35-56, g/d	22.38±3.62^{b}	23.31±3.80^{b}	28.93±3.97^{a}	0.026
DBWG 57-70, g/d	40.71±5.03	43.66±5.29	40.95±5.12	0.288
DBWG 71-90, g/d	29.28±4.71^{b}	36.16±4.42^{a}	31.53±4.85^{b}	0.006
DBWG 35-90, g/d	29.6±1.4^{b}	33.2±1.4^{a}	32.9±1.5^{a}	<0.001
FCR, g/g	3.65±0.11	3.62±0.10	3.64±0.11	0.698
Mortality, %	0	0	0	1.000

SEM= standard error of the mean; BW= body weight; DBWG= daily body weight gains; FCR= feed conversion ratio.

^{a,b,c} Values with different superscripts are significantly different at $P<0.05$.

Hot carcass weight with head differed between the groups and ranged from 1,437.7 g in SPM group to 1,356.0 g in MLM group and 1,270.8 g in the control group, where rabbits were fed without silkworm and mealworm larvae (Table 5). SPM group was characterized by higher dressing percentage compared to C and MLM groups (59.88 % *vs* 57.67 % and 57.49 %). Weights of livers ranged from 74.33 g in C group to 79.83 g in MLM group, however, differences in this feature were not statistically significant. No significant differences were observed between the groups in the weights of digestive

tract. There were no differences in the amount of inguinal, shoulder and perirenal fat, either. Carcass muscle weight was higher in SPM and MLM groups compared to C group (1,092.5 g and 10,56.7 g *vs* 914.9 g).

Table 5: Carcass characteristic of rabbits (mean±SEM)

	Group			***P***
	Control	**SPM**	**MLM**	
Hot carcass weight with head, g	1,270.8±17.1[c]	1,437.7±22.6[a]	1,356.0±17.3[b]	<0.001
Dressing percentage	57.67±0.30[b]	59.88±0.61[a]	57.49±0.036[b]	0.002
Liver weight, g	74.33±3.85	76.00±1.29	79.83±3.00	0.165
Weight of heart, kidneys and lungs, g	42.50±2.81	43.33±1.54	44.16±2.01	0.853
Weight of digestive tract, g	424.16±10.11	477.16±26.88	498.33±22.38	0.064
Weight of inguinal fat, g	4.66±0.84	4.33±0.49	4.83±0.40	0.841
Weight of shoulder fat, g	12.50±1.78	11.67±0.61	12.33±0.92	0.212
Weight of perirenal fat, g	14.16±1.53	15.66±2.78	15.83±1.53	0.188
Carcass muscle weight, g	914.9±17.1[b]	1092.5±45.2[a]	1056.7±14.5[a]	0.001

SEM= standard error of the mean.

[a,b,c] Values with different superscripts are significantly different at $P<0.05$.

Analysis of the basic chemical composition of hind leg and loin muscles revealed no between-group differences in the amount of dry matter, ash and crude protein (Table 6). Protein content in hind leg muscles ranged from 21.85 % in the control rabbits to 22.10 % in rabbits supplemented with silkworm pupae meal. Protein content in loin muscles was slightly higher and varied from 22.95 % in MLM group to 23.37 % in SPM group. Crude ash content ranged from 1.19 to 1.21 % in hind leg muscles and from 1.22 to 1.25 % in loin. Proportion of ether extract in hind leg muscles did not differ significantly between groups, however, a tendency towards a higher share of this compound in the experimental groups than in the control group was observed (2.06 % in SPM and 2.25 % in MLM groups *vs* 1.72 % in C group). In turn, in loin muscles, content of ether extract was significantly higher in rabbits belonging to MLM group than in those from C group (1.90 *vs* 1.22 %).

Table 6: Proximate chemical composition (% of fresh matter) of hind leg and loin muscles of rabbits (mean±SEM)

	Group			*P*
	Control	**SPM**	**MLM**	
Hind leg muscles				
Dry matter	23.84±0.17	24.51±0.27	24.42±0.30	0.161
Crude ash	1.20±0.01	1.19±0.01	1.21±0.01	0.192
Crude protein	21.85±0.15	22.10±0.17	21.88±0.10	0.442
Ether extract	1.72±0.12	2.06±0.20	2.25±0.30	0.061
Loin muscles				
Dry matter	25.28±0.26	24.90±0.16	24.87±0.17	0.304
Crude ash	1.23±0.02	1.22±0.01	1.25±0.01	0.525
Crude protein	22.98±0.20	23.37±0.16	22.95±0.20	0.246
Ether extract	1.22±0.06[b]	1.37±0.10[a]	1.90±0.37[a]	0.038

SEM= standard error of the mean.

[a,b] Values with different superscripts are significantly different at $P<0.05$.

Discussion

The control diet contained 10 % of SB, and the experimental diets (SPM and MLM) contained 5 % of soybean meal, and 4 % of silkworm pupae or 4 % of mealworm larvae meals, respectively. It was found that partial replacement of soybean meal with the experimental components improved the weight gains and body weights of rabbits, but had no effect on FCR. These results correspond with the ether extract content and energy value of the diets. However, the higher proportion of ether extract in the diets with insect meals, especially with mealworm larvae, increased the share of this compound in rabbit's muscles.

Similar results to ours, in terms of the body weights of NZW rabbits, were observed earlier[20]. Similar DBWG in NZW rabbits, although calculated for the age range of 30-80 d, were also reported[21]. NZW rabbits involved in this experiment belongs to the average productive genetic line. Although they grow slightly more rapidly than equally popular California broiler rabbits[22], commercially bred hybrid rabbits may reach over 3000 g on d 84 of age[8,23].

The protein content was determined to be 187.1 g/kg in the control diet and 187.7 g/kg and 187.6 g/kg in the experimental diets. However, both silkworm pupae and mealworm larvae contain chitin. It is a polysaccharide composed of acetylglucosamine (N-acetyl-D-glucose-2-amine) mers. The nitrogen in mers increases the level of protein in laboratory analyses. Silkworm pupae contain 3-4% chitin[24]. Chitin content in mealworm larvae is around 5 %[25]. It may reduce nutrient digestibility[26], but the

present experiment showed that chitin had no adverse effect on FCR. It is worth mentioning that chitin may have health beneficial properties. Chitin is not degraded in the small intestine and it can be fermented by the microbiota of the large intestine. It is suggested that chitin may restore the compositional balance of the microbial community. In addition, chitin, or derivate, seems to exhibit anti-viral, anti-tumour, antifungal activities and antimicrobial properties and a bacteriostatic effect on pathogenic bacteria[26].

The range of DP obtained in this study was higher than that reported by other Polish authors[20,27]. Particular attention should be given to liver weight, the high value of which may suggest that the diet places an excessive burden on the animal's body. Certain between-group differences were observed in liver weights of the rabbits (ranging from 74.33 g in C group to 79.83 g in MLM group), but within-group differences were too high to show a significant effect of the diet on liver weight. The analysis of liver weight against carcass weight shows the ratios of 5.85 %, 5.29 % and 5.89 % for successive rabbit groups (data not shown in table). Because the highest liver weight in MLM rabbits corresponds with the amount of dietary fat and the amount of carcass fat, at this stage it is difficult to conclude whether mealworm larvae added to the diet have a negative impact on the health of rabbits. This aspect should be continuously investigated in future, more extensive research. It is of interest to note, however, that in a previous experiment performed in the same pavilion with the same line of NZW rabbits, rabbits fed with 5% of corn DDGS had an average liver weight of 95.8 g[20].

The obtained content of dry matter, protein, ash and ether extract in meat is characteristic of broiler rabbits. Similar protein and ash contents of rabbit meat to those observed in the present study were also reported by other authors[27,28,29]. Intramuscular fat is one of the major determinants of sensory meat quality. The amounts of fat obtained in our study seem typical for NZW rabbits, which are slaughtered at around 90 days of age. It should be noted, however, that use of insect's meals in rabbits feeding increased the amount of ether extract in their muscles. Lower ranges of fat (0.27 to 0.33 %) were noted by Daszkiewicz *et al*[27], whereas Chełmińska and Kowalska[20] found fat content to be 2.31 % in the control group, 3.72 % in the group receiving 5 % of DDGS, and as much as 4.94 % in the group fed with 10 % of DDGS.

Conclusions and implications

Partial replacement of soybean meal with silkworm pupae and mealworm larvae meals in rabbit diets may improve the body weight and body weight gains of rabbits, together with some carcass characteristic, without influencing FCR. The use of insects as a source of feed for rabbits neither has an effect on the content of dry matter, crude protein and crude ash, but increases the amount of ether extract in their muscles.

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