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Effect of dietary soybean meal replacement with a combination of white lupine seeds and insect meals on the growth performance, carcass traits, and meat quality of rabbits

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Abbreviated title: Rabbits fed diets with insects

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Abstract

The aim of this study was to determine the effect of complete dietary replacement of soybean meal (SBM) with combinations of silkworm pupae meal and white lupine seeds (SPM+WLS), mealworm larvae meal and white lupine seeds (MLM+WLS), and Zophobas larvae meal and white lupine seeds (ZLM+WLS) on the growth performance of rabbits, carcass dressing percentage, the proportion of primal cuts in the carcass, the content of selected compounds in the loin, and the concentrations of short-chain fatty acids (SCFAs) in the large intestine. A total of 80 New Zealand White rabbits at 35 days of age were divided into four feeding groups. The control diet (group C) contained 10% SBM. In experimental groups, SBM was completely replaced with SPM+WLS, MLM+WLS, and ZLM+WLS (5% + 5%). Complete replacement of SBM with the above combinations of protein sources had no negative effect on the growth performance of rabbits. The loin of group C rabbits had the highest DM content, the lowest EE content, the lowest proportion of total polyunsaturated fatty acids (PUFAs), and the highest levels of retinol and α-tocopherol. The concentrations of SCFAs in the large intestine were higher in rabbits fed diets containing SBM than in their counterparts fed diets supplemented with insect meals and white lupine seeds. This indicates that SBM in rabbit diets can be replaced by a combination of the above components, but further research is needed to optimize the proportion of insect meals and white lupine seeds in rabbit diets and evaluate their effect on the health status and performance of animals.

Key words: rabbit feeding, silkworm pupae meal, mealworm larvae meal, Zophobas larvae meal, short-chain fatty acids

In intensive farming systems, rabbits are fed complete diets containing up to 18% protein. Soybean meal (SBM) is the most common source of dietary protein for broiler rabbits (Purwin

et al. 2019). However, in some regions, particularly those where soybeans are not grown, a recent trend has emerged of eliminating SBM from livestock diets. Consequently, efforts are being made to avoid importing SBM and to address concerns regarding the feeding of genetically modified products to farm animals (Tudisco et al. 2006). Therefore, alternative dietary protein sources are being tested. Edible insects are a rich source of high-quality protein. Insects grow relatively quickly and reproduce easily. Approximately 2 kg of feed biomass are required to produce 1 kg of insects (Collavo et al., 2005). Insect meals are characterized by high nutritional value, and their protein content varies between 40% and 60%, depending on the growth stage and species (Makkar et al., 2014). Most insects, including Coleoptera, Lepidoptera, Hymenoptera, and Dipterazanim, undergo complete metamorphosis and pass through four main stages in their life cycle: the egg, the larva, the pupa, and the imago. Some other insects, such as Hemiptera and Acrididae, undergo incomplete metamorphosis that consists of only three main stages: the egg, the nymph, and the imago. Insect larvae are most often used as animal feed, followed by pupae and imagines. Examples include black soldier fly (*Hermetia illucens*) larvae (Diener et al., 2011), house fly (*Musca domestica*) larvae (Calvert et al., 1969), mealworm (*Tenebrio molitor*) larvae (Klasing et al., 2000; Bovera et al., 2016), Zophobas (*Zophobas morio*) larvae (Józefiak et al. 2020), silkworm (*Bombyx mori*) pupae (Donatti, 1992), and locust and cricket imagines (Kierończyk et al., 2018). In rabbit nutrition, edible insects are a source of both protein and fat (Dalle Zotte et al. 2020, Gasco et al. 2020, Kowalska et al. 2020, Cullere et al. 2022, Volek et al. 2023).

Legume seeds, including white lupine seeds, are yet another source of protein in rabbit diets. Wild white lupine is an annual plant that is widespread in the Mediterranean Region, where it has been cultivated since ancient times. At present, white lupine is cultivated in Eurasia, Africa, and both Americas. White lupine seed production in Europe has increased from 88 000 tons in 2000 to 458 000 tons in 2020 (Pereira 2022). In 2020, the leading European lupine producers were Poland (261 000 tons), followed by Germany (34 000 tons), France (13 000 tons), and Spain (2 000 tons) (FAOSTAT 2022). This plant species has a high yield potential, which in Poland reaches 3 to 4 tons of seeds per hectare. White lupine seeds contain 30-35% protein and can be a valuable protein component of rabbit feed. However, they also contain antinutritional compounds, mainly alkaloids (Bastianelli et al. 1998, Chilomer et al. 2010), and may have a negative effect on animal health when fed in large quantities. In contrast, research has shown that white lupine seeds, similarly to other legume seeds, have no adverse effects on rabbits when fed in moderate amounts (Gugołek et al. 2018, Uhlířová et al. 2018, Volek et al. 2020, Šufliarský et al. 2024).

The scientific literature provides data on the efficacy of insect larvae and white lupine seeds in livestock nutrition, but only when they are used separately. However, it can be hypothesized that insect meals combined with white lupine seeds may be a viable alternative to SBM in rabbit diets. In view of the above, the aim of this study was to determine the effect of complete dietary replacement of SBM with combinations of silkworm pupae meal and white lupine seeds (SPM+WLS), mealworm larvae meal and white lupine seeds (MLM+WLS), and Zophobas larvae meal and white lupine seeds (ZLM+WLS) on the growth performance of rabbits, carcass dressing percentage, the proportion of primal cuts in the carcass, the content of selected compounds in the loin, and the concentrations of short-chain fatty acids (SCFAs) in the large intestine.

Material and methods

Animals and treatments

Eighty New Zealand White growing rabbits raised in a closed facility on a farm in southern Poland were used in the present study. The animals were divided into four equal groups (n=20), analogous in terms of origin, sex ratio, and body weight (BW). The experiment began when rabbits were weaned at 35 d of age and ended when they reached 90 d of age. Rabbits were housed in wire net flat-deck cages (0.5 x 0.6 x 0.4 m; 1 animal per cage), 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 a regulated photoperiod (16 h of light and 8 h of darkness).

The control diet (group C) contained 10% SBM. In experimental groups, SBM was completely replaced with SPM+WLS, MLM+WLS, and ZLM+WLS (5% + 5%). Dried silkworm pupae and dried mealworm and Zophobas larvae were purchased on a local market, and they were ground three times to obtain meal. White lupine seeds cv. "Kulig" were used in this study. Antinutritional factors were not removed from any of the tested feed ingredients. The chemical composition and energy value of the major dietary components are presented in Table 1, the ingredient composition of rabbit diets is presented in Table 2, and the chemical composition and energy value of diets are presented in Table 3.

Table 1. Chemical composition (% of fresh matter) and measured energy content (MJ/kg) of soybean meal (SBM), white lupine seeds (WLS), silkworm pupae meal (SPM), mealworm larvae meal (MLM), and Zophobas larvae meal (ZLM)

Specification	Component				
	SBM	WLS	SPM	MLM	ZLM
Dry matter	88.01	88.23	94.15	94.53	96.36
Crude ash	6.32	3.75	3.89	3.42	3.58
Crude protein	46.16	36.46	51.32	47.52	41.00
Ether extract	1.57	8.42	26.38	33.53	39.36
NDF	12.53	17.74	5.47	10.85	10.15
ADF	7.30	14.11	8.31	6.59	7.17
ADL	0.64	0.93	2.49	2.15	2.08
Lysine	2.75	1.65	2.63	3.02	2.93
Methionine + cystine	1.46	0.91	1.48	3.98	3.85
Threonine	1.68	1.21	1.73	2.44	2.32
Tryptophan	0.63	0.24	0.40	0.80	0.74
Gross energy	17.45	18.58	24.53	26.16	26.97

NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin.

Table 2. Ingredients of rabbit diets (%)

Ingredient	Group			
	Control	SPM+WLS	MLM+WLS	ZLM+WLS

Soybean meal (SBM)	10	-	-	-
Silkworm pupae meal (SPM)	-	5	-	-
Mealworm larvae meal (MLM)	-	-	5	-
Zophobas larvae meal (ZLM)	-	-	-	5
White lupine seeds (WLS)	-	5	5	5
Wheat bran	27	27	27	27
Alfalfa meal	26	26	26	26
Barley	16.5	18	18	18
Dried beet pulp	10	10	10	10
Corn	6	6	6	6
Rapeseed oil	1.5	-	-	-
Salt	0.2	0.2	0.2	0.2
Ground limestone	1.3	1.3	1.3	1.3
Feed phosphate	0.5	0.5	0.5	0.5
Vitamin-mineral premix	1	1	1	1

Table 3. Chemical composition (% of fresh matter) and measured energy content (MJ/kg) of rabbit diets

Specification	Group			
	Control	SPM+WLS	MLM+WLS	ZLM+WLS
Dry matter	89.03	89.16	89.08	89.17
Crude ash	7.57	8.32	8.44	8.40
Crude protein	15.97	15.96	15.78	15.53
Ether extract	3.86	4.06	4.27	4.52
NDF	23.27	23.43	24.37	24.29
ADF	11.93	12.81	12.20	12.43
ADL	2.71	3.02	2.95	2.91
Lysine	0.74	0.71	0.70	0.69
Methionine + cystine	0.46	0.45	0.59	0.56
Threonine	0.58	0.55	0.58	0.56
Tryptophan	0.21	0.19	0.20	0.19
Gross energy	16.47	16.50	16.55	16.58

WLS - white lupine seeds, SPM - silkworm pupae meal, MLM - mealworm larvae meal, ZLM - Zophobas larvae meal, NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin.

Experimental procedures

The animals had ad libitum access to feed administered once daily via automatic feeders and water from nipple drinkers. All animals were allowed to practice cecotrophy.

The rabbits were weighed on an electronic scale on days 35 and 90. The results were used to calculate their daily body weight gains (BWG g/d) and the feed conversion ratio (FCR) [feed intake (g) / BWG (g)].

At the end of the feeding trial, the animals were fasted for 24 h to more precisely determine their BW. Then the rabbits were sacrificed according to the standard guidelines for euthanizing experimental animals. Carcasses were skinned and eviscerated. Samples of the large intestine were collected to determine the concentrations of selected organic acids. Loin samples were collected from the carcasses to determine the levels of cholesterol, collagen, retinol, and α-tocopherol. Carcasses were chilled for 24 hours at 0–3°C, and carcass dressing percentage was calculated as follows:

$$\frac{\text{weight of an eviscerated carcass}}{\text{animal's live weight}} \text{ x } 100$$

The carcasses were divided into the head (cut through the craniovertebral joint), the fore part (cut between the seventh and eighth thoracic vertebrae), the loin (cut between the sixth and seventh thoracic vertebrae), and the hind part (carcass section remaining after the separation of the loin from the front, comprising the hindquarters and hind limbs) (Daszkiewicz et al. 2012). Then, loin samples were collected for chemical analyses. The saturation index (S/P) was calculated for fatty acids in the loin, using the equations proposed by Peiretti and Meineri (2008) and Volek and Marounek (2009): S/P = (C14:0 + C16:0 + C18:0) / ∑MUFAs + ∑PUFAs.

Chemical analyses

The chemical composition of feed and animal muscles was determined by the official methods of analysis of AOAC International (AOAC 2006). All analyses were carried out in duplicate. Dry matter (DM) content was determined in a laboratory drier, at 103°C. Crude ash (CA) content was estimated by sample mineralization in a muffle furnace (Czylok, Poland) at 600°C. Total nitrogen content was determined by the Kjeldahl method, in the FOSS TECATOR Kjeltec 2200 Auto Distillation Unit. Ether extract (EE) content was estimated by the Soxhlet method, in the FOSS SOXTEC SYSTEM 2043.

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed in the FOSS TECATOR Fibertec 2010 System; NDF was determined as described by Van Soest et al. (1991), and ADF was determined using the official methods of analysis of AOAC International (AOAC 2006). The levels of amino acids (AOAC method 982.30) were determined using the Biochrom 20 plus amino acid analyzer and Biochrom amino acid analysis reagents (Biochrom Ltd., Cambridge, England). Gross energy content was determined in a bomb calorimeter (IKA® C2000 basic, Germany).

To determine fatty acid composition, all fat samples were methylated by the modified Peisker method (1.5 cm^3 of a methanol:chloroform:concentrated sulfuric acid mixture, 100:100:1 v/v, was added to ca. 150 µl fat, thermostat – 80ºC, 3h), and fatty acid methyl esters were obtained. Fatty acids were separated and determined by gas chromatography: VARIAN CP–3800 gas chromatograph-Netherlands, flame-ionization detector (FID), capillary column (length – 50 m, ϕ = 0.25 mm, film d = 0.25µm), split injector, split ratio 50:1, 1 µl sample, detector temperature – 250ºC, injector temperature – 225ºC, column temperature – 200ºC, carrier gas – helium, flow rate – 1.2 cm^3 /min. Fatty acids were identified by comparing the

retention times of individual fatty acid methyl ester standards (Sigma-Aldrich) and the retention times of peaks in the analyzed samples. The relative content of each fatty acid was expressed as a percentage of the total peak area of all fatty acids in the sample. Cholesterol levels were determined in samples of fat extracted by the standard Folch method (Rhee et al., 1982), using POINTE SCIENTIFIC test kits that employ enzyme systems (horse radish peroxidase).

The concentrations of retinol and α-tocopherol in the loin were determined as described by Hőgberg et al. [55] and Xu [56]. One gram loin samples were homogenized with 1.2 cm^3 of 20% ascorbic acid in H_2O (Ultra-Turrax T25 homogenizer, IKA, Staufen, Germany) and saponified (375 g KOH, 750 cm^3 H_2O, 450 cm^3 methanol, 80°C, 15′). Ethanol (35% v/v) and NaCl (10% w/v) were added to cooled samples, which were extracted twice with n-hexane (2 × 4 cm3) and centrifuged (MPW-350R centrifuge, Poland, 1500 g, 15′). Next, 3 cm^3 and 4 cm^3 samples of the supernatant were collected successively. The combined extracts were evaporated to dryness under a stream of nitrogen. The residue was dissolved in 1 cm^3 of 96% ethanol to determine the content of tocopherols and retinol. The levels of retinol and α-tocopherol were determined by reversed-phase high-performance liquid chromatography (RP-HPLC system, SHIMADZU, Kyoto, Japan), on a Nucleosil C18 column, with methanol/water (95:5, *v*:*v*) as the mobile phase, a UV detector (326 nm for retinol) and a fluorescence detector (excitation–295 nm, emission–330 nm for tocopherol), flow rate: 1 $cm^3\,min^{-1}$, loop: 20 μl. The concentrations of retinol and tocopherol were estimated by comparing their peak areas with those of external standards (Sigma-Aldrich, Darmstadt, Germany: (±)-α-tocopherol–No Cat. T3251, retinol-vitamin A–alcohol-No Cat. R7632; the recoveries of α-tocopherol and retinol were 96% ± 2.8% and 89% ± 4.5%, respectively).

Collagen concentration was determined according to the official methods of analysis of AOAC International (AOAC 2006), method 990.26.

Statistical analysis

Data were expressed as means and the standard errors of the mean (SEM). The results were processed statistically using one-way analysis of variance (ANOVA), and the significance of differences between groups was determined by Duncan's multiple range test. The calculations were performed using Statistica™ (StatSoft® Inc. 2015) software.

Results

The growth performance and carcass characteristics of rabbits are presented in Table 4. The final BW of rabbits was higher in group MLM+WLS than in group ZLM+WLS. In turn, BWG (g/d) was higher in group C and in group MLM+WLS than in group ZLM+WLS. Carcass dressing percentage was higher in group C than in group ZLM+WLS, whereas no significant differences in the proportion of primal cuts in the carcass were found between the groups. The carcasses of group C rabbits had the significantly highest DM content of the loin and the lowest EE content of the loin.

Table 4. Growth performance and carcass characteristics of rabbits

Specification	Group				P-value
	Control	SPM+WLS	MLM+WLS	ZLM+WLS	

BW at 35 days of age(g)	773.9±18.69	781.4±17.37	778.5±17.27	782.2±18.25	0.982
BW at 91 days of age (g)	2488.4±68.23	2483.7±73.92	2511.7±81.84[a]	2403.7±64.86[b]	0.037
BWG (g/d)	30.62±1.42[a]	30.40±1.36	30.95±1.47[a]	29.18±1.31[b]	0.045
FCR (g/g gain)	3.62±0.10	3.58±0.11	3.66±0.13	3.71±0.12	0.251
Mortality (%)	0	0	0	0	1.000
DP (%)	57.73±0.47[a]	57.63±0.38	56.97±0.42	56.38±0.40[b]	0.024
Fore part (%)	36.27±0.17	35.86±0.15	35.65±0.17	36.14±0.16	0.130
Loin (%)	26.73±0.12	26.80±0.13	27.17±0.14	26.85±0.13	0.538
Hind part (%)	37.00±.016	37.34±0.16	37.18±0.17	37.01±0.15	0.621
DM (%) in loin	28.90±0.86[a]	26.61±0.20[b]	27.30±0.28[b]	26.54±0.26[b]	0.021
CA (%) in loin	1.14±0.01	1.16±0.01	1.14±0.01	1.15±0.01	0.440
CP (%) in loin	23.16±0.44	23.40±0.36	23.24±0.32	23.54±0.34	0.606
EE (%) in loin	2.56±0.30[b]	2.68±0.23	2.93±0.37[a]	3.13±0.26[a]	0.039

WLS - white lupine seeds, SPM - silkworm pupae meal, MLM - mealworm larvae meal, ZLM - Zophobas larvae meal, BW – body weight, BWG – body weight gain, FCR – feed conversion ratio, DP – dressing percentage, DM – dry matter, CA- crude ash, CP – crude protein, EE – ether extract.
a, b – values marked with different superscript letters in a row differ significantly ($P \leq 0.05$).

The content of selected compounds in the loin of rabbits is presented in Table 5. Rabbits from groups C and MLM+WLS were characterized by higher concentrations of essential amino acids in the loin than rabbits from groups SPM+WLS and ZLM+WLS. Methionine concentration in the loin was highest in rabbits fed diets supplemented with MLM+WLS and lowest in animals from group C and group SPM+WLS, and the noted differences were significant. Tryptophan concentration in the loin was highest in group C, lower in groups SPM+WLS and MLM+WLS, and lowest in group ZLM+WLS. The concentrations of fatty acids in the loin also varied significantly between the groups. The proportion of total saturated fatty acids (SFAs) in the loin was highest in group C and lowest in group SPM+WLS (control > MLM+WLS and ZLM+WLS > SPM+WLS). Palmitoleic acid concentration was higher in group C than in group ZLM+WLS. Oleic acid concentration was lowest in group C. The proportion of total monounsaturated fatty acids (MUFAs) in the loin did not differ significantly between the groups. Linoleic acid concentration was highest in group ZLM+WLS, lower in group MLM+WLS, and lowest in groups C and SPM+WLS. α-linolenic acid concentration was higher in group SPM+WLS than in the other three groups. In turn, arachidonic acid concentration was highest in groups ZLM+WLS and SPM+WLS, and significantly lower in group C. The proportion of total polyunsaturated fatty acids (PUFAs) in the loin was higher in groups SPM+WLS and ZLM+WLS than in groups C and MLM+WLS. The 6/3 PUFA ratio varied across the groups. The highest value of this ratio was observed in group ZLM+WLS, followed by group MLM+WLS and group C, and its lowest value was found in group SPM+WLS. The S/P ratio was higher in group C than in group SPM+WLS. The cholesterol content of the loin was significantly higher in groups SPM+WLS and C, and significantly lower in group ZLM+WLS. The concentrations of retinol and α-tocopherol in the loin were highest in group C, where retinol concentration was higher than in group MLM+WLS, and α-

tocopherol concentration was higher than in group SPM+WLS. No differences in the collagen content of the loin were observed between the groups, and its concentration ranged from 0.56 to 0.65 g/100g.

Table 5. Content of selected compounds in the loin of rabbits

Specification	Group				P-value
	Control	SPM+WLS	MLM+WLS	ZLM+WLS	
Lysine	9.38±0.19	9.11±0.14	8.94±0.19	9.09±0.06	*0.307*
Methionine	2.89±0.08^{b}	2.93±0.06^{b}	3.21±0.04^{a}	3.04±0.11	*0.045*
Cystine	1.22±0.06	1.16±0.03	1.12±0.05	1.15±0.03	*0.504*
Threonine	4.70±0.04	4.67±0.06	4.79±0.07	4.63±0.03	*0.206*
Tryptophan	1.36±0.02^{a}	1.14±0.05^{b}	1.10±0.03^{b}	0.90±0.02^{c}	*<0.001*
Total essential amino acids	38.82±0.37^{a}	37.54±0.21^{b}	38.84±0.50^{a}	37.78±0.26^{b}	*0.029*
Total SFAs	42.23±0.47^{a}	38.40±0.40^{c}	39.77±0.21^{b}	40.14±0.24^{b}	0.002
C16:1 (palmitoleic)	6.96±0.56^{a}	6.21±0.91	6.86±0.77	4.40±0.88^{b}	0.029
C18:1 (oleic)	28.46±0.26^{b}	30.05±0.24^{a}	29.65±0.25^{a}	30.14±0.23^{a}	0.006
Total MUFAs	35.43±0.73	36.28±1.16	36.53±0.98	34.61±0.96	0.507
C18:2 (linoleic)	15.84±0.26^{c}	15.87±0.64^{c}	17.05±0.81^{b}	18.14±0.64^{a}	0.041
C18:3 (α-linolenic)	5.08±0.20^{b}	6.95±0.49^{a}	4.47±0.17^{b}	4.27±0.16^{b}	<0.001
C20:4 (arachidonic)	1.21±0.34^{b}	2.08±0.19^{a}	1.91±0.21	2.56±0.40^{a}	0.038
Total PUFAs	22.32±0.35^{b}	25.31±0.92^{a}	23.69±1.14^{b}	25.24±0.87^{a}	0.042
6/3 PUFAs	3.31±0.17^{c}	2.53±0.17^{d}	4.12±0.11^{b}	4.67±0.21^{a}	<0.001
S/P	0.72±0.06^{a}	0.61±0.06^{b}	0.65±0.05	0.66±0.06	0.018
Cholesterol (mg/g)	0.60±0.02^{a}	0.62±0.02^{a}	0.58±0.01	0.56±0.01^{b}	0.040
Retinol (μg/g)	0.21±0.04^{a}	0.15±0.03	0.15±0.01^{b}	0.19±0.00	0.032
α-tocopherol (μg/g)	5.25±0.83^{a}	3.92±0.52^{b}	4.36±0.17	4.66±0.21	0.026
Collagen (g/100g)	0.62±0.06	0.56±0.04	0.65±0.06	0.57±0.03	0.594

WLS - white lupine seeds, SPM - silkworm pupae meal, MLM - mealworm larvae meal, ZLM - Zophobas larvae meal, SFAs – saturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids.

a, b, c, d – values marked with different superscript letters in a row differ significantly (P≤0.05).

The concentrations of the majority of the analyzed SCFAs (propionic, butyric, isovaleric, and valeric acids) in the large intestine of rabbits (Table 6) were highest in group C, lower in groups SPM+WLS and MLM+WLS, and lowest in group ZLM+WLS (control > SPM+WLS and MLM+WLS > ZLM+WLS). In addition, acetic acid concentration in the large intestine was higher in group C than in groups MLM+WLS and ZLM+WLS. Isobutyric acid concentration in the large intestine was higher in groups C and MLM+WLS, and lower in groups SPM+WLS and ZLM+WLS.

Table 6. Concentrations of short-chain fatty acids (μmol/g) in the large intestine of rabbits

Acid	Group	P-value

	Control	SPM+WLS	MLM+WLS	ZLM+WLS	
Acetic	60.97±8.95[a]	50.30±3.25	46.07±7.40[b]	43.84±4.26[b]	0.038
Propionic	5.38±0.55[a]	4.08±0.28[b]	3.87±0.59[b]	3.22±0.46[c]	0.007
Isobutyric	0.21±0.03[a]	0.16±0.01[b]	0.22±0.04[a]	0.15±0.01[b]	0.042
Butyric	19.75±3.25[a]	16.33±1.06[b]	15.14±2.17[b]	12.79±1.72[c]	<0.001
Isovaleric	0.41±0.04[a]	0.34±0.02[b]	0.36±0.05[b]	0.32±0.02[c]	0.004
Valeric	1.19±0.11[a]	0.81±0.07[b]	0.75±0.06[b]	0.61±0.06[c]	<0.001

WLS - white lupine seeds, SPM - silkworm pupae meal, MLM - mealworm larvae meal, ZLM - Zophobas larvae meal.

a, b, c – values marked with different superscript letters in a row differ significantly (P≤0.05).

Discussion

Previous studies have shown that the use of insect larvae/pupae meals and white lupine seeds may be a feasible alternative to SBM in rabbit diets. For instance, Kowalska et al. (2020) demonstrated that the addition of silkworm pupae or mealworm larvae meals increased carcass weight and PUFA-3 content in muscles. Strychalski et al. (2021) and Gugołek et al. (2021) reported that such an addition improved fat and fiber digestibility and induced beneficial changes in gut microbiota. Volek and Marounek (2009) and Volek et al. (2023) confirmed the absence of negative effects of white lupine seeds on growth performance and digestibility, as well as their favorable impact on carcass yield and the reduction of the sanitary risk index. However, the question of the effect exerted by these dietary components when used in combination remained unanswered. In the authors' opinion, the approach based on integrating dietary components, as opposed to testing them separately, supports their practical application because all nutritionally balanced diets consist of several ingredients. To the best of the authors' knowledge, this is the first study to investigate the use of a combination of insect larvae/pupae meals and white lupine seeds in rabbit nutrition. As shown in Tables 1 and 3, insect meal compensates for the deficiencies of white lupine seeds in terms of protein content and amino acid composition, providing rabbits with a complete protein blend. However, both insect meal and lupine seeds contain certain quantities of antinutritional factors. Chitin is the predominant antinutritional compound found in insect meal. Silkworm pupae contain 3–4% chitin (Suresh et al. 2012), whereas the chitin content of mealworm and Zophobas larvae is around 5% (Song et al. 2018, Soon et al. 2018). Chitin may reduce nutrient digestibility, but it may also play a health-promoting role in restoring the compositional balance of the gut microbial community. In addition, the bacteriostatic, antiviral, antitumor, and antifungal properties of this substance have been postulated (Piccolo et al. 2017, Wijesekara 2024). In lupine seeds, the primary antinutritional factors are alkaloids (mainly quinolizidine alkaloids), of which approximately 76% is lupanine. However, while seeds of the wild forms of *L. albus* contain up to 12.73% of alkaloids in DM, their content in modern cultivars is as low as 0.02% (Kroc et al. 2017). Alkaloids administered in small amounts exert antimutagenic, antibacterial, antifungal, anticancer, and anti-inflammatory effects (Pereira et al. 2022). In addition, lupanine improves glucose homeostasis in the body, due to its anti-diabetic potential (Wiedemann et al. 2015). Insect meals and white lupine seeds in appropriate doses have emerged as a promising alternative to SBM in rabbit diets (Cullere et al. 2024, Šufliarský et al. 2024), which can contribute to their diversification.

To the best of the authors' knowledge, the efficacy of Zophobas larvae in rabbit nutrition has not been analyzed in the literature to date. As shown in Table 4, group ZLM+WLS rabbits were characterized by lower final BW and daily BWG than their counterparts from group MLM+WLS. In addition, the FCR was slightly higher in group ZLM+WLS than in the remaining groups, which may indicate lower feed efficiency in the former group, although the noted differences were not significant. These results seem surprising because, as demonstrated by previous research, ZLM added to diets of other animal species (chickens, fish, and pigs) tended to exert a positive influence on their growth parameters (Alves et al. 2020, Józefiak et al. 2020, Prachom et al. 2021, Rumbos et al. 2021). However, the results of studies investigating other animal species cannot be directly extrapolated to rabbits because the dietary inclusion levels of ZLM varied widely, ranging from 0.2% in chickens to 30% in Nile tilapia. The above research findings do not provide a basis for determining the optimal amount of ZLM in the diet, which should be approached with caution. The proportion of primal cuts in the carcass did not differ significantly between the groups. The content of selected compounds such as lysine, threonine, cystine, essential amino acids, and collagen in rabbit meat did not differ significantly between groups ZLM+WLS and C. It should be stressed that the fatty acid profile of rabbit meat in group ZLM+WLS was desirable from the consumer's point of view due to its high proportion of total PUFAs.

Vitamins A and E are non-enzymatic components of the antioxidant defense system. One of the major forms of vitamin A is retinol, and α-tocopherol is the most biologically active form of vitamin E. The levels of retinol and α-tocopherol in the loin were highest in group C (Table 5), but these results should be interpreted in view of the relatively lowest EE content noted in this group (Table 4). Higher levels of retinol (7.26 μg/g) and α-tocopherol (7.82 μg/g) in the loin of rabbits were recorded in a previous study (Strychalski et al. 2016). In the experiment conducted by Lo Fiego et al. (2004), the α-tocopherol content of rabbit loin was 2.10 μg/g, whereas Sulli et al. (1998) did not detect α-tocopherol in the triceps muscle of rabbits. Such differences should be interpreted with caution. In the case of vitamin E, discrepancies between test results may be due to the differences in its dietary levels, forms, bioavailability, and assay methods as well as the health status of animals. The age of the tested rabbits is another important consideration. In the current study, rabbits were examined at 91 days of age, whereas those analyzed by Strychalski et al. (2016) were 160 days old.

This is the first study to investigate the effect of diets supplemented with insect meals on the production of SCFAs in the large intestine of rabbits. The concentrations of most fatty acids in the large intestine were highest in group C and lowest in group ZLM+WLS (Table 6). Short-chain fatty acids are synthesized primarily from dietary fiber that is not digested in the small intestine. They are an important source of energy for rabbits and contribute to maintaining gut health, including the growth of gut microbiota and intestinal barrier function (Bellier and Gidenne 1996, Peinheiro et al. 2009). Therefore, the proportion of SCFAs is one of the indicators of the health status of rabbits. From this perspective, rabbits fed diets containing SBM appear to be healthier than their counterparts fed diets supplemented with insect meals and WLS. However, it had no effect on the final BW of rabbits (Table 4), which is determined by digestive health (Fortun-Lamothe and Boullier 2007, Combes et al. 2013). It should be noted that mortality cases were not recorded in any of the groups.

Conclusions

Complete replacement of SBM with combinations of insect meals and white lupine seeds had no negative effect on the growth performance or mortality of rabbits. No significant differences in protein or collagen content were found between meat from rabbits fed diets containing combinations of the tested ingredients and meat from group C animals. The loin of group C rabbits had the highest DM content, the lowest EE content, the lowest proportion of total PUFAs, and the highest levels of retinol and α-tocopherol. In addition, the concentrations of SCFAs in the large intestine were higher in rabbits fed diets containing SBM than in their counterparts fed diets supplemented with insect meals and white lupine seeds. This indicates that SBM in rabbit diets can be replaced by a combination of the above components, but further research is needed to optimize the proportion of insect meals and white lupine seeds in rabbit diets and evaluate their effect on the health status and performance of animals.

References

Alves A.P.C., Paulino R.R., Pereira R.T., Costa D.V., Rosa P.V. (2020). Nile tilapia fed insect meal: growth and innate immune response in different times under lipopolysaccharide challenge. Aquac. Res., 52: 529–540.

AOAC. (2006). Association of Official Analytical Chemists. Official methods of analysis. 18th ed. Arlington (VA): Association of Analytical Communities.

Bastianelli D., Grosjean F., Peyronnet C., Duparque M., Regnier J. M. (1998). Feeding value of pea (Pisum sativum, L.) 1. Chemical composition of different categories of pea. Anim. Sci., 67: 609–619.

Bellier R., Gidenne T. (1996). Consequences of reduced fibre intake on digestion, rate of passage and caecal microbial activity in the young rabbit. Br. J. Nutr., 75: 353–363.

Bovera F., Loponte R., Marono S., Piccolo G., Parisi G., Iaconisi V., Gasco L., Nizza A. (2016). Use of larvae meal as protein source in broiler diet: Effect on growth performance, nutrient digestibility, and carcass and meat traits. J. Anim. Sci., 94: 639–647.

Calvert C.C., Martin R.D., Morgan N.O. (1969). Housefly pupae as food for poultry. J. Econ. Entomol., 62: 938–939.

Chilomer K., Zaleska K., Ciesiolka D., Gulewicz P., Frankiewicz A., Gulewicz, K. (2010). Changes in the alkaloid, alpha-galactoside and protein fractions content during germination of different lupin species. Acta Soc. Bot. Poloniae, 79(1): 11–20.

Collavo A., Glew R.H., Huang Y.S., Chuang L.T., Bosse R., Paoletti M.G. (2005). House cricket small-scale farming. In: Ecological implications of minilivestock: potential of insects, rodents, frogs and snails, 27, 515–540. Science Publiscers Inc., Enfield, New Hampshire.

Combes S., Fortun-Lamothe L., Cauquil L., Gidenne T. (2013). Engineering the rabbit digestive ecosystem to improve digestive health and efficacy. Animal, 7: 1429–1439.

Cullere M., Singh Y., Gerencsér Z., Matics Z., Cappellozza S., Dalle Zotte A. (2022). Silkworm (*Bombyx mori* L.) oil in growing rabbit nutrition: effects on meat physicochemical traits, sensory profile and shelf-life. J. Insects Food Feed, 8: 733–742.

Cullere M., Szendrő Z., Matics Z., Gerencsér Z., Singh Y., Dalle Zotte A. (2024). Effect of silkworm (*Bombyx mori* L.) pupae oil dietary inclusion on growth performance, digestibility and carcase traits of growing rabbits. Ital. J. Anim. Sci., 23: 751–757.

Dalle Zotte A., Singh Y., Gerencsér Z., Matics Z., Szendrő Z., Cappellozza S., Cullere, M. (2022). Feeding silkworm (*Bombyx mori* L.) oil to growing rabbits improves the fatty acid composition of meat, liver and perirenal fat. Meat Sci., 193: 108944.

Daszkiewicz T., Gugołek A., Janiszewski P., Kubiak D., Czoik M. (2012). The effect of intensive and extensive production systems on carcass quality in New Zealand White rabbits. World Rabbit Sci. 20: 25–33.

Diener S., Zurbrügg C., Roa Gutiérrez F., Nguyen D.H., Morel A., Koottatep T., Tockner K. (2011). Black soldier fly larvae for organic waste treatment – prospects and constraints. Proc 2nd International Conference on Solid Waste Management in the Developing Countries, Khulna, Bangladesh, 52–59.

Donatti F.C. (1992). Utilization of silkworm pupae meal (Bombyx mori L.) as a source of protein in the diet of growing-finishing pigs. R. Soc. Bras. Zootec., 21: 378–383.

Food and Agriculture Organization of the United Nations (FAO) FAOSTAT. (2022). Available online: https://www.fao.org/faostat/en/#home (accessed on 15 November 2024).

Fortun-Lamothe L., Boullier S. (2007). A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. Livest. Sci., 107: 1–18.

Gasco L., Biancarosa I., Liland N.S. (2020). From waste to feed: A review of recent knowledge on insects as producers of protein and fat for animal feeds. Curr. Opin. Green Sust. Chem., 23: 67–79.

Gugołek A., Juśkiewicz J., Kowalska D., Zwoliński C., Sobiech P., Strychalski J. (2018). Physiological responses of rabbits fed with diets containing rapeseed meal, white lupine and pea seeds as soybean meal substitutes. Cienc. Agrotec., 42: 297–306.

Gugołek A., Kowalska D., Strychalski J., Ognik K., Juśkiewicz J. (2021). The effect of dietary supplementation with silkworm pupae meal on gastrointestinal function, nitrogen retention and blood biochemical parameters in rabbits. BMC Vet. Res., 17: 204.

Högberg A., Pickova J., Babol J., Andersson K., Dutta P.C. (2002). Muscle lipids, vitamins E and A, and lipid oxidation as affected by diet and RN genotype in female and castrated male Hampshire crossbreed pigs. Meat Sci., 60: 411–420.

Józefiak A., Benzertiha A., Kierończyk B., Łukomska A., Wesołowska I., Rawski M. (2020). Improvement of cecal commensal microbiome following the insect additive into chicken diet. Animals, 10: 577.

Kierończyk B., Rawski M., Józefiak A., Mazurkiewicz J., Świątkiewicz S., Siwek M., Bednarczyk M., Szumacher-Strabel M., Cieślak A., Benzertiha A., Józefiak D. (2018). Effects of replacing soybean oil with selected insect fats on broilers. Anim. Feed Sci. Technol., 240: 170–183.

Klasing K.C., Thacker P., Lopez M.A., Calvert C.C. (2000). Increasing the calcium content of mealworms (Tenebrio molitor) to improve their nutritional value for bone mineralization of growing chicks. J. Zoo Wild. Medic. 31: 512–517.

Kowalska D., Gugołek A., Strychalski J. (2020). Evaluation of slaughter parameters and meat quality of rabbits fed diets with silkworm pupae and mealworm larvae meals. Ann. Anim. Sci., 20: 551–564.

Kroc M., Rybiński W., Wilczura, P., Kamel K., Kaczmarek Z., Barzyk P., Święcicki W. (2017). Quantitative and qualitative analysis of alkaloids composition in the seeds of a white lupin (*Lupinus albus* L.) collection. Genet. Resour. Crop Evol., 64: 1853–1860.

Lo Fiego D.P., Santoro P., Macchioni P., Mazzoni D., Piattoni F., Tassone F., De Leonibus E. (2004). The effect of dietary supplementation of vitamins C and E on the α-tocopherol content of muscles, liver and kidney, on the stability of lipids, and on certain meat quality parameters of the longissimus dorsi of rabbits. Meat Sci., 67: 319–327.

Makkar H.P.S., Tran G., Heuzé V., Ankers P. (2014). State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol., 197: 1–33.

Peiretti P.G., Meineri G. (2008). Effects on growth performance, carcass characteristics, and the fat and meat fatty acid profile of rabbits fed diets with chia (*Salvia hispanica* L.) seed supplements. Meat Sci., 80: 1116–1121.

Pereira A., Ramos F., Sanches Silva A. (2002). Lupin (*Lupinus albus* L.) Seeds: Balancing the Good and the Bad and Addressing Future Challenges. Molecules, 27: 8557.

Pinheiro V., Guedes C.M., Outor-Monteiro D., Mourão J.L. (2009). Effects of fibre level and dietary mannanoligosaccharides on digestibility, caecal volatile fatty acids and performances of growing rabbits. Anim. Feed Sci. Technol., 148: 288–300.

Piccolo G., Iaconisi V., Marono S., Gasco L., Loponte R., Nizza S., Bovera F., Parisi G. (2017). Effect of Tenebrio molitor larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (Sparus aurata). Anim. Feed Sci. Technol., 226: 12–20.

Prachom N., Boonyoung S., Hassaan M.S., El-Haroun E., Davies S.J. (2021). Preliminary evaluation of Superworm (*Zophobas morio*) larval meal as a partial protein source in experimental diets for juvenile Asian sea bass, *Lates calcarifer*. Aquac. Nutr., 27: 1304–1314.

Purwin C., Gugołek A., Strychalski J., Fijałkowska M. (2019). Productivity, nutrient digestibility, nitrogen retention, and meat quality in rabbits fed diets supplemented with *Sida hermaphrodita*. Animals, 9: 901.

Rhee K.S., Dutson T.R., Smith G.C., Hostetler R.L., Reiser R. (1982). Cholesterol content of raw and cooked beef longissimus muscles with different degrees of marbling. J. Food Sci., 47: 716–719.

Rumbos C.I., Athanassiou C. (2021). The Superworm, *Zophobas morio* (Coleoptera: Tenebrionidae): A 'Sleeping Giant' in Nutrient Sources. J. Insect Sci., 21: 13.

Song Z., Li G., Guan F., Liu W. (2018). Application of chitin/chitosan and their derivatives in the papermaking industry. Polymers, 10: 389.

Soon C.Y., Tee Y.B., Tan C.H., Rosnita A.T., Khalina A. (2018). Extraction and physicochemical characterization of chitin and chitosan from Zophobas morio larvae in varying sodium hydroxide concentration. Int. J. Biol. Macromol., 108: 135–142.

StatSoft Inc. (2015). Statistica (data analysis software system), Version 12.

Strychalski J., Gugołek A., Antoszkiewicz Z., Kowalska D., Konstantynowicz M. (2016). Biologically active compounds in selected tissues of white-fat and yellow-fat rabbits and their production performance parameters. Livest. Sci., 183: 92–97.

Strychalski J., Juśkiewicz J., Kowalska D., Gugołek A. (2021). Performance indicators and gastrointestinal response of rabbits to dietary soybean meal replacement with silkworm pupae and mealworm larvae meals. Arch. Anim. Nutr., 75: 294–310.

Šufliarský P., Volek Z., Ebeid T.A., Zita L. (2024). Dietary inclusion of white lupine seeds and their byproducts in rabbits can contribute to EU agricultural sustainability: a review. World Rabbit Sci., 32: 145–160.

Sulli K.C., Sun J., Giraud D.W., Moxley R.A., Driskell J.A. (1998). Effects of β-carotene and α-tocopherol on the levels of tissue cholesterol and triglyceride in hypercholesterolemic rabbits. J. Nutr. Biochem., 9: 344–350.

Suresh H.N., Mahalingam C.A., Pallavi. (2012). Amount of chitin, chitosan and chitosan based on chitin weight in pure races of multivoltine and bivoltine silkworm pupae Bombyx mori L. Int. J. Sci. Nat. 3: 214–216.

Tudisco R., Lombardi P., Bovera F., d'Angelo D., Cutrignelli M.I., Mastellone V., Terzi V., Avallone L., Infascelli F. (2006). Genetically modified soya bean in rabbit feeding: detection of DNA fragments and evaluation of metabolic effects by enzymatic analysis. Anim. Sci. 82: 193–199.

Uhlířová L., Volek Z., Marounek M. (2018). White lupin bran and its effects on the growth performance, carcass characteristics and digestibility of nutrients in fattening rabbits. World Rabbit Sci., 26(1): 1–6.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991). Methods for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583–3597.

Volek Z., Marounek M. (2009). Whole white lupin (*Lupinus albus* cv. Amiga) seeds as a source of protein for growing-fattening rabbits. Anim. Feed Sci. Technol., 152: 322–329.

Volek Z, Uhlířová L, Zita L. (2020). Narrow-leaved lupine seeds as a dietary protein source for fattening rabbits: a comparison with white lupine seeds. Animal, 14(4): 881–888.

Volek Z., Zita L., Adámková A., Adámek M., Mlček J., Plachý V. (2023). Dietary inclusion of crickets (*Acheta domesticus*) and yellow mealworm meal (*Tenebrio molitor*) in comparison with soybean meal: effect on the growth, total tract apparent digestibility, and nitrogen balance of fattening rabbits. Animals, 13: 1637.

Wiedemann M., Gurrola-Díaz C.M., Vargas-Guerrero B., Wink M., García-López P.M., Düfer M. (2015). Lupanine Improves Glucose Homeostasis by Influencing KATP Channels and Insulin Gene Expression. Molecules, 20(10): 19085–19100.

Wijesekara T., Baojun X. (2024). New Insights into Sources, Bioavailability, Health-Promoting Effects, and Applications of Chitin and Chitosan. J. Agric. Food Chem., 72: 17138–17152.

Xu Z. (2008). Comparison of extraction methods for quantifying vitamin E from animal tissues. Bioresour. Technol. 99: 8705–8709.

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