Original

The effect of fish and mealworm larvae meals as alternative dietary protein sources on nutrient digestibility and gastrointestinal function in *Chinchilla lanigera*

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Abstract: Chinchillas are herbivores, but wild chinchillas may occasionally consume animal-based foods. The aim of this study was to determine the effect of fish meal (FM) and mealworm meal (MWM) included in complete pelleted diets on nutrient digestibility and gastrointestinal function in chinchillas. The experiment was performed on 24 male, divided into three groups, n=8. Control group (C) was fed a diet containing 10% soybean meal (SBM). In the experimental group FM, chinchillas received a diet containing 3% fish meal, and the diet administered to the experimental group MWM was supplemented with 4% dried mealworm larvae meal. The nutrient digestibility of diets was determined. At the end of the experiment animals were euthanized and their digestive tracts were removed to analyze gut activity. FM group animals were characterized by lower crude fat digestibility, whereas both alternative protein sources improved the digestibility of acid detergent fiber (ADF). A considerable increase in the activity of cecal intracellular and extracellular bacterial enzymes (in particular β-glucosidase, β-galactosidase and β-xylosidase) was noted in the FM group, which however did not increase the concentrations of short-chain fatty acids (SCFA). The inclusion of MWM in chinchilla diets shifted the bacterial fermentation site from the cecum (lowest SCFA pool) to the colon (highest SCFA pool), thus enabling to derive additional energy from less digestible dietary components. In conclusion, chinchilla diets can be supplemented with small amounts of animal protein such as fish meal and dried mealworm larvae meal.
Key words: *Chinchilla lanigera*, fish meal, gut activity, mealworm larvae meal, nutrient digestibility

Introduction

Chinchillas (*Chinchilla lanigera*) are small rodents native to South America. They are almost extinct in the wild, and their wild colonies can be found only in Chilean reserves [32]. Around the world, chinchillas are farm-raised for fur, kept as laboratory and pet animals [7, 19, 24, 33]. Chinchillas are obligate herbivores. The structure and function of a chinchilla's digestive tract are complex. Similarly to other rodents, chinchillas have voluminous colons and ceca, which contributes to their ability to digest fibrous feed. The adaptation known as a colonic separation mechanism is responsible for the accumulation of microorganisms in their ceca, leading to the formation of re-ingested cecotropes [12, 23, 26, 29]. Hirakawa [11] described the digestive process in the chinchilla as a species that practices coprophagy.

Nutrient digestibility in the chinchilla remains insufficiently investigated in comparison with other rodents. The few studies addressing this issue have been con-

ducted by Rogier [25], Krishnamurti *et al.* [17] and Głogowski *et al.* [7], while Hagen *et al.* [10] analyzed mean digesta retention times in the chinchilla.

Little is known about the food habits of wild chinchillas. Interestingly, according to older references, wild chinchillas eat insects and bird eggs when they are available [13]. The authors' breeding experience and the information found on websites created by pet chinchilla owners also indicate that chinchillas willingly consume feeds of animal origin in small quantities. However, a different opinion was expressed by Cortes *et al.* [3] who studied the food habits of the chinchillas.

Fish meal is one of animal protein sources in chinchilla diets. Commercial pelleted diets for chinchillas, particularly those manufactured in Western Europe, usually contain small amounts of fish meal as a source of amino acids improving fur quality. However, this practice has not been documented in the scientific literature. Edible insects are increasingly used in animal nutrition. There has been a growing interest in various insect species as potential dietary protein sources. Dietary supplementation with dried mealworm (*Tenebrio molitor*) larvae has already been tested in fish, pigs and broiler chickens [2, 6, 14].

The efficacy of animal protein sources in chinchilla nutrition has not been researched to date. Thus, the present study was undertaken to fill the knowledge gap in this area. The aim of this experiment, performed on chinchillas, was to determine the effect of fish meal and mealworm larvae meal included in complete pelleted diets on nutrient digestibility and gastrointestinal function, with particular emphasis on microbial fermentation processes in the large gut.

Materials and Methods

Ethical declaration

The animal protocol and the number of animals used in this study complied with the guidelines of the respective Polish national legislations, according to European Union standards on animal experimentation and care of animals under study.

Animals, diets and management

The experiment was performed on 24 male standard chinchillas (*Chinchilla lanigera*) at 248 ± 23 days of age, divided into three groups (n=8) that were equal in terms of origin and body weight. The animals were fed *ad libitum* complete pelleted diets, prepared at the laboratory of the Department of Fur-bearing Animal Breeding and Game Management, University of Warmia and Mazury in Olsztyn (Poland). Feed pellets were 4 mm in

diameter and 6 mm in length. Control group (C) chinchillas were fed a diet containing 10% soybean meal (SBM). In the first experimental group (FM), chinchillas received a diet containing 3% fish meal, and the diet administered to the second experimental group (MWM) was supplemented with 4% dried mealworm larvae meal. All diets were isonitrogenous and contained more than 18% total protein. Diet composition is shown in Table 1, and the chemical composition of diets and experimental factors is presented in Table 2. The chemical composition of feed was similar to that of the basal diet described by Głogowski *et al.* [7].

The experiment was carried out on a large commercial chinchilla farm in north-eastern Poland, in June and July. The animals were housed in a separate facility, under standard environmental conditions: temperature, 16–18°C; relative humidity, 65%; controlled photoperiod (12 h light, 25 lx, and 12 h dark), in individual wire-mesh flat-deck cages measuring $0.40 \times 0.45 \times 0.34$ m, equipped with automatic feeders and nipple drinkers. The cages had wire mesh floors and containers for the collection of feces and lost feed pellets.

Data and sample collection

At the beginning and at the end of the feeding trial, chinchillas were weighed on an electronic scale within an accuracy of 1 g. Average daily gains were determined,

Table 1. Dietary ingredients (%)

Ingredients	Diet		
	C	FM	MWM
Soybean meal	10.0	7.0	6.0
Fish meal	0.0	3.0	0.0
Dried mealworm larvae meal	0.0	0.0	4.0
Dried alfalfa	25.0	25.0	25.0
Ground wheat	15.0	15.0	15.0
Wheat bran	22.0	22.0	22.0
Rapeseed meal	4.0	4.0	4.0
Corn DDGS[a]	4.0	4.0	4.0
Arbocel[b]	4.0	4.0	4.0
Dried beet pulp	11.8	11.8	11.8
Dried brewer's yeast	0.5	0.5	0.5
Dried whey	1.0	1.0	1.0
Salt	0.2	0.2	0.2
Calcium phosphate	1.5	1.5	1.5
Mineral-vitamin premix[c]	1.0	1.0	1.0
Total	100.0	100.0	100.0

C, control; FM, fish meal; MWM, mealworm larvae meal. [a]Dried distillers grains with solubles. [b]Crude fiber concentrate. [c]Composition of mineral-vitamin premix (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 × 5H2O, 24,5%), 800 mg; Co, 1,000 mg; I, 100 mg; Ca, 150 g; P, 100 g.

Table 2. Chemical composition of diets and experimental factors in fresh matter (%)

	Diet			Soybean meal	Fish meal	Mealworm larvae meal
	C	FM	MWM			
Dry matter	92.04	92.44	92.05	89.55	93.63	93.39
Crude ash	7.20	7.54	7.62	5.92	12.01	6.95
Organic matter	84.84	84.90	84.43	83.63	81.38	86.44
Total protein	18.09	18.61	18.23	46.52	60.11	50.35
Crude fat	2.81	3.07	3.78	2.03	10.17	27.92
NDF	27.09	26.81	27.12	12.33	0.02	11.36
ADF	17.69	17.38	17.73	6.11	1.60	7.41
ADL	5.18	5.11	6.61	0.68	1.89	3.12
Lysine	0.76	0.80	0.76	3.01	4.32	2.98
Methionine+cystine	0.52	0.61	0.51	1.29	2.16	1.08
Threonine	0.68	0.68	0.69	1.76	1.97	2.16
Tryptophan	0.16	0.15	0.15	0.65	0.57	0.59
Gross energy [MJ/kg]	16.51	16.84	16.99	17.38	18.86	23.87

C, control; FM, fish meal; MWM, Mealworm larvae meal; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignine.

and average daily feed intake was calculated as the difference between feed offered and leftovers.

During the 10-day digestibility trial, feed intake and leftovers were recorded, and feces samples were collected for analyses. The apparent digestibility coefficients (ADC) of nutrients were calculated as $ADC = a − b/a × 100\%$, where a and b denote the nutrient content of diets and feces, respectively.

The entire experiment lasted 36 days (including a 20-day adaptation period and a 10-day digestibility trial proper, followed by 6 days when chinchillas were fed experimental diets, until slaughter). The animals were euthanized by electrical current at over 9 months of age ($292 ± 23$ days), which is the standard slaughter age on the farm where the experiment was conducted.

The animals were skinned, and gastrointestinal segments were removed (stomach, small intestine, cecum and colon) as soon as possible after slaughter (20–30 min). Samples of fresh gastric and intestinal digesta were analyzed to determine: pH, viscosity, dry matter content, and the concentrations of ammonia and short-chain fatty acids (SCFA).

Sample analysis

The content of dry matter (method 934.01), crude ash (method 942.05), total protein (N × 6.25; method 976.05), crude fat (method 920.39), neutral detergent fiber (NDF) (method 2002.04), acid detergent fiber (ADF) (method 989.03) and acid detergent lignin (ADL) (method 973.18) was determined according to AOAC [1]. The content of ADF and NDF was assayed with heat-stable amylase and expressed inclusive of residual ash. ADL content was determined using sulfuric acid. The levels of amino acids, lysine, methionine, cystine, threonine and tryptophan, in diets were determined using

the Biochrom 20 plus amino acid analyzer and Biochrom amino acid analysis reagents (Biochrom Ltd., Cambridge, UK). Gross energy content was determined using a bomb calorimeter (IKA® C2000 basic, Staufen, Germany).

The remaining cecal digesta samples were transferred to microcentrifuge tubes and were stored at −70°C until analyses of bacterial enzymatic activity. The pH of gastric, jejunal, cecal and colonic digesta was measured with the use of a microelectrode and a pH/ION meter (model 301, Hanna Instruments, Vila do Conde, Portugal). The activity of jejunal mucosal sucrase, maltase and lactase was assayed by a previously described method [34]. The amount of released glucose was measured spectrophotometrically, and enzyme activity was expressed in μmol of disaccharide hydrolyzed per min and in grams of protein. Pooled samples of small intestinal digesta were collected, vortexed and centrifuged at 7,211 g for 10 min. The supernatant fraction (0.5 ml) was placed in the Brookfield LVDV-II+ cone-plate rotational viscometer (CP40; Brookfield Engineering Lab., Stoughton, MA, USA), and the viscosity of pooled samples was measured at a constant temperature of 37°C and a shear rate of 60/s. Viscosity was recorded as apparent viscosity. Dry matter concentrations in the jejunal and cecal digesta were determined after drying the samples at 103°C. Ammonia was extracted from fresh cecal digesta, trapped in a solution of boric acid in Conway dishes, and determined as previously reported [34].

Cecal and colonic SCFA concentrations were determined by gas chromatography (Shimadzu GC-2010, Kyoto, Japan). The samples (0.2 g) were mixed with 0.2 ml of formic acid, diluted with deionized water and centrifuged at 7,211 g for 10 min. The supernatant was loaded onto a capillary column (SGE BP21, 30 m × 0.53

mm) using an on-column injector. The initial oven temperature was 85°C, it was raised to 180°C at 8°C min-1 and held there for 3 min. The temperature of the flame ionization detector and the injector was 180°C and 85°C, respectively. The sample volume for GC analysis was 1 μl. The concentrations of cecal and colonic putrefactive SCFA (PSCFA) were calculated as the sum of iso-butyric acid, iso-valeric acid and valeric acid. All SCFA analyses were performed in duplicate. Pure acetic, propionic, butyric, iso-butyric, iso-valeric and valeric acids were obtained from Sigma Co. (Poznań, Poland), and their mixture was used to create a standard plot and then to calculate the amount of individual acids. This additional set of pure acids was included in each GC run of samples at five sample intervals to maintain calibration.

Cecal fermentation processes were analyzed based on the activity of selected bacterial enzymes (α-and β-glucosidase, α- and β-galactosidase, β-glucuronidase, α-arabinopyranosidase, β-xylosidase), measured by the rate of release of p-nitrophenol or o-nitrophenol from the respective nitrophenylglucosides, according to a previously described method [9]. The following substrates were used: p-nitrophenyl-α-D-glucopyranoside (for α-glucosidase), p-nitrophenyl-β-D-glucopyranoside (for β-glucosidase), p-nitrophenyl-α-D-galactopyranoside (for α-galactosidase), o-nitrophenyl-β-D-galactopyranoside (for β-galactosidase), p-nitrophenyl-β-D-glucuronide (for β-glucuronidase), p-nitrophenyl-α-L-arabinopyranosidase (for α-arabinopyranosidase), p-nitrophenyl-β-D-xylopyranoside (for β-xylosidase). In order to measure the activities of enzymes secreted by bacterial cells into the cecal environment, a reaction mixture was prepared containing 0.3 ml of a substrate solution (5 mM) and 0.2 ml of a 1:10 (v/v) dilution of the cecal sample in 100 mM phosphate buffer (pH 7.0) after centrifugation at 7,211 g for 15 min. Incubation was carried out at 37°C, and p-nitrophenol was quantified at 400 nm (o-nitrophenol concentration - at 420 nm) after the addition of 2.5 ml of 0.25 M-cold sodium carbonate. Enzyme activity was expressed as μmol product formed per h per g of digesta. In order to determine the total activity of selected cecal bacterial enzymes, including extracellular activity (see the procedure above) and intracellular activity, a cecal digesta sample diluted in phosphate buffer was mechanically disrupted by vortexing with glass beads (212–300 μm in diameter; four periods of 1 min with 1 min cooling intervals on ice) using the FastPrep®-24 homogenizer (MP Biomedicals, Santa Ana, CA, USA). The resulting mixture was centrifuged at 7,211 g for 15 min at 4°C. The supernatant was used for the enzyme assay described above. Intracellular enzyme activity was calculated by comparing total

enzyme activity with the activities of bacterial enzymes secreted into the intestinal environment, and it was expressed as μmol product (PNP or ONP, p-nitrophenol or o-nitrophenol, respectively) formed per h per g of digesta. In order to prepare the calculation formulas, the model curves for PNP and ONP (PNP or ONP standard solution in a 100 mM phosphate buffer pH 7.0, 40 mg/l) were used and appropriate equations were derived. Extracellular enzyme activity was determined as the rate of enzyme release, expressed as a percentage of total enzyme activity. All analyses were performed in duplicate.

Statistical analysis

The results were analyzed statistically using one-way analysis of variance (ANOVA) at a significance level of $P<0.05$. When significant treatment effects were found, post-hoc comparisons were performed using Duncan's multiple range test. Data are expressed as mean values and SEM. Calculations were performed with Statistica 12.0 [30].

Results

The mortality rate, body weight of chinchillas, average daily gain and average daily feed intake are presented in Table 3. Mortality cases were not recorded during the study, and experimental diets did not cause visible signs of gastrointestinal disorders in chinchillas. No significant differences were found in the average body weight of animals determined at the beginning and at the end of the experiment. However, the average final body weight of chinchillas fed diets supplemented with mealworm larvae meal (MWM) was somewhat higher, but not statistically significant, than the final body weight of control group animals (C) and those receiving fish meal (FM) (by 27 g and 21 g, respectively). Average daily gains were highest in group MWM ($P=0.038$ vs. C and FM). Average daily feed intake was similar in all groups (over 17 g).

Nutrient and energy digestibility values in chinchillas are shown in Table 4. No significant differences in the digestibility coefficients of dry matter, organic matter, total protein, NDF and gross energy were observed between groups. It should be noted, however, that total protein digestibility was higher ($P=0.055$) in groups C and MWM than in group FM. The digestibility coefficient of crude fat was highest in chinchillas fed diets supplemented with mealworm larvae meal ($P=0.001$ vs. C and FM). The digestibility coefficient of ADF was similar in experimental groups FM and MWM, and significantly higher than in group C ($P=0.023$). Highly

Table 3. Growth performance of chinchillas (mean ± SEM)

	Diet			*P*-value
	C	FM	MWM	
Mortality rate [%]	0 ± 0	0 ± 0	0 ± 0	–
Initial body weight [g]	586.3 ± 8.99	586.4 ± 11.12	589.9 ± 9.36	0.958
Final body weight [g]	597.7 ± 11.16	603.7 ± 15.32	624.70 ± 8.50	0.165
Average daily gain [g/day]	0.317 ± 0.184[b]	0.480 ± 0.280[b]	0.966 ± 0.094[a]	0.038
Average daily feed intake [g/day]	17.32 ± 0.99	17.47 ± 0.92	17.56 ± 0.87	0.983

C, control; FM, fish meal; MWM, Mealworm larvae meal. [a,b]Mean values within rows with no common superscripts are different at *P*<0.05.

Table 4. Nutrient and energy digestibility (%) (mean ± SEM)

	Diet			*P*-value
	C	FM	MWM	
Dry matter	66.57 ± 0.75	65.10 ± 1.17	66.32 ± 0.57	0.451
Organic matter	69.38 ± 0.70	68.21 ± 1.06	69.10 ± 0.53	0.558
Total protein	78.60 ± 0.75	74.98 ± 1.32	77.17 ± 0.90	0.055
Crude fat	89.56 ± 0.65[b]	87.36 ± 0.84[b]	92.17 ± 0.51[a]	0.001
NDF	34.62 ± 1.82	36.82 ± 2.18	36.13 ± 1.02	0.663
ADF	32.27 ± 1.94[b]	39.26 ± 2.45[a]	39.34 ± 1.26[a]	0.023
ADL	33.52 ± 2.59[b]	37.87 ± 2.75	44.38 ± 1.37[a]	0.010
Gross energy	67.57 ± 0.63	67.11 ± 1.09	68.24 ± 0.59	0.613

C, control; FM, fish meal; MWM, Mealworm larvae meal; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignine. [a,b]Mean values within rows with no common superscripts are different at *P*<0.05.

Table 5. Selected gastrointestinal tract parameters in chinchillas (mean ± SEM)

	Diet			*P*-value
	C	FM	MWM	
Stomach				
pH of digesta	3.62 ± 0.158[b]	4.95 ± 0.237[a]	4.07 ± 0.228[b]	<0.001
Small intestine				
Tissue weight [g/kg BW]	7.25 ± 0.253	7.74 ± 0.310	7.05 ± 0.184	0.085
Digesta weight [g/kg BW]	13.6 ± 0.864	16.2 ± 1.228[a]	12.8 ± 0.301[b]	0.020
Viscosity [mPa·s]	3.89 ± 0.282[a]	4.21 ± 0.140[a]	2.91 ± 0.126[b]	<0.001
DM of jejunal digesta [%]	17.9 ± 0.614[a]	16.1 ± 0.655[b]	18.1 ± 0.561[a]	0.036
pH of jejunal digesta	7.25 ± 0.050	7.03 ± 0.099	7.15 ± 0.068	0.068
Sucrase [μmol/min/g protein]	9.59 ± 1.351[a]	9.27 ± 0.636[a]	6.31 ± 0.865[b]	0.039
Maltase [μmol/min/g protein]	18.4 ± 0.884[a]	18.3 ± 1.270[a]	13.2 ± 1.200[b]	0.006
Lactase [μmol/min/g protein]	1.62 ± 0.157[a]	1.72 ± 0.194[a]	1.17 ± 0.141[b]	0.036
Cecum				
Tissue weight [g/kg BW]	6.09 ± 0.311[a]	4.97 ± 0.453[b]	4.77 ± 0.169[b]	0.015
Digesta weight [g/kg BW]	36.3 ± 1.606[a]	29.3 ± 2.049[b]	27.8 ± 2.162[b]	0.008
DM of digesta [%]	18.5 ± 0.709	17.5 ± 0.853	18.1 ± 0.544	0.339
Ammonia [mg/g]	0.145 ± 0.018[b]	0.198 ± 0.023[a]	0.132 ± 0.010[b]	0.023
pH of cecal digesta	7.54 ± 0.017	7.47 ± 0.023	7.54 ± 0.061	0.383
Colon				
Colon with digesta	51.1 ± 2.292	50.1 ± 4.470	60.5 ± 4.434	0.087
Weight of ascending colonic tissue [g/kg BW]	10.1 ± 0.131[b]	10.3 ± 0.416	11.0 ± 0.205[a]	0.031
Weight of ascending colonic digesta [g/kg BW]	15.1 ± 0.435[b]	19.5 ± 2.522	23.5 ± 1.899[a]	0.006
pH of ascending colonic digesta	7.67 ± 0.086	7.70 ± 0.077	7.49 ± 0.063	0.073

C, control; FM, fish meal; MWM, Mealworm larvae meal; BW, body weight. [a,b]Mean values within rows with no common superscripts are different at *P*<0.05.

significant differences in ADL digestibility were observed between groups MWM and C (*P*=0.010).

Table 5 presents gastrointestinal tract parameters in chinchillas. The pH of gastric digesta was significantly increased by dietary FM treatment (*P*<0.001 vs. C and MWM). The highest relative weight of the small intestinal contents was noted in chinchillas fed the FM diet, and it was significantly different from the values found

Table 6. Bacterial enzyme activity in the cecal digesta of chinchillas[1] (mean ± SEM)

	Diet			P-value
	C	FM	MWM	
α-Glucosidase				
Extracellular	8.55 ± 0.961^a	11.6 ± 1.450^a	7.41 ± 0.501^b	0.013
Intracellular	9.29 ± 0.935	10.9 ± 1.595	7.75 ± 0.487	0.070
Total	17.8 ± 1.874	22.5 ± 2.978^a	15.2 ± 0.857^b	0.028
Extracellular[2]	47.6 ± 0.941^b	52.0 ± 1.437^a	48.8 ± 1.666^{ab}	0.049
β-Glucosidase				
Extracellular	8.99 ± 0.960^b	12.5 ± 0.904^a	8.82 ± 1.183^b	0.025
Intracellular	9.93 ± 0.698^b	13.1 ± 0.668^a	10.4 ± 1.142^b	0.023
Total	18.9 ± 1.624^b	25.6 ± 1.359^a	19.2 ± 2.313^b	0.022
Extracellular[2]	47.0 ± 1.376	48.7 ± 1.518	45.4 ± 0.977	0.107
α-Galactosidase				
Extracellular	30.2 ± 3.627	37.9 ± 3.984^a	25.9 ± 2.382^b	0.028
Intracellular	33.9 ± 3.117	42.9 ± 4.927^a	28.7 ± 2.531^b	0.016
Total	64.1 ± 6.718	80.8 ± 8.552^a	54.5 ± 4.658^b	0.018
Extracellular[2]	46.7 ± 0.915	47.2 ± 1.648	47.2 ± 0.982	0.778
β-Galactosidase				
Extracellular	48.5 ± 3.068^b	67.9 ± 5.884^a	42.9 ± 4.644^b	0.002
Intracellular	43.0 ± 1.979^b	57.9 ± 3.491^a	37.6 ± 5.155^b	0.002
Total	91.5 ± 4.686^b	126 ± 9.154^a	80.5 ± 9.409^b	0.001
Extracellular[2]	52.8 ± 1.131	53.6 ± 1.010	53.8 ± 1.982	0.649
β-Glucuronidase				
Extracellular	43.3 ± 3.919	50.0 ± 9.166	56.4 ± 6.658	0.220
Intracellular	51.9 ± 3.959	58.3 ± 10.37	63.5 ± 8.474	0.346
Total	95.1 ± 7.774	108 ± 19.31	120 ± 14.49	0.272
Extracellular[2]	45.3 ± 0.811	46.0 ± 1.532	47.2 ± 1.889	0.418
α-Arabinopyranosidase				
Extracellular	6.40 ± 0.568	6.86 ± 0.896	6.58 ± 0.733	0.687
Intracellular	7.17 ± 0.499^b	10.1 ± 0.698^a	6.89 ± 0.720^b	0.003
Total	13.6 ± 1.063	17.0 ± 0.980	13.5 ± 1.412	0.055
Extracellular[2]	46.9 ± 0.651	40.1 ± 3.861^b	48.6 ± 1.258^a	0.025
β-Xylosidase				
Extracellular	6.09 ± 0.682^b	7.85 ± 0.424^a	5.13 ± 0.612^b	0.005
Intracellular	6.67 ± 0.866^b	8.58 ± 0.532^a	5.67 ± 0.633^b	0.011
Total	12.8 ± 1.504^b	16.4 ± 0.808^a	10.8 ± 1.228^b	0.005
Extracellular[2]	48.1 ± 1.578	47.8 ± 1.636	47.4 ± 1.143	0.747

C, control; FM, fish meal; MWM, Mealworm larvae meal. [1]μmol/h/g digesta. [2]Expressed as percentage of total (extra- + intracellular) enzymatic activity. [a,b]Mean values within rows with no common superscripts are different at $P<0.05$.

in animals fed the MWM diet. The viscosity of small intestinal digesta decreased significantly in response to the MWM treatment ($P<0.001$ vs. C and FM). Dietary supplementation with fish meal led to a decrease in the dry matter content of jejunal digesta ($P=0.036$ vs. C and MWM). The lowest activities of jejunal mucosal sucrase, maltase and lactase were noted in the MWM treatment ($P=0.039$, $P=0.006$ and $P=0.036$, respectively). Both alternative dietary protein sources incorporated into chinchilla diets caused a significant decrease in the relative weights of cecal tissue and digesta, compared with the control dietary treatment. Cecal ammonia concentration increased in response to the FM diet ($P=0.023$ vs. C and MWM). The relative weights of ascending colonic tissue and digesta increased in the MWM treatment, compared with group C ($P=0.031$ and $P=0.006$, respectively).

Bacterial enzyme activity in the cecal digesta is presented in Table 6. The highest extracellular and total activities of bacterial α-glucosidase in the cecal digesta were observed in the FM group ($P=0.013$ vs. MWM). The release rate of α-glucosidase from bacterial cells into the cecal environment, expressed in terms of extracellular activity as a percentage of total (extracellular and intracellular) activity, was highest in the FM treatment. When compared with C and MWM treatments, a significant increase in the extracellular, intracellular and total activities of bacterial β-glucosidase, β-xylosidase and β-galactosidase in the cecal digesta was noted in chinchillas fed the FM diet. The MWM treatment resulted in the lowest (extracellular, intracellular and total) cecal activity of bacterial α-galactosidase (in all cases $P<0.05$ vs. FM). The intracellular activity of bacterial α-arabinopyranosidase was significantly enhanced in

Table 7. Concentration, profile and pool of SCFA[1] in the cecal and colonic digesta of chinchillas (mean ± SEM)

	Diet			P-value
	C	FM	MWM	
Cecum				
SCFA concentrations [μmol/g digesta]				
acetic	22.0 ± 0.887	20.7 ± 2.707	20.2 ± 0.851	0.498
propionic	4.02 ± 0.345	3.78 ± 0.301	3.57 ± 0.279	0.342
iso-butyric	0.450 ± 0.040	0.466 ± 0.070	0.390 ± 0.019	0.304
butyric	3.72 ± 0.325	3.38 ± 0.374	3.24 ± 0.281	0.342
iso-valeric	0.406 ± 0.031	0.440 ± 0.087	0.275 ± 0.022	0.057
valeric	0.341 ± 0.021[a]	0.343 ± 0.026[a]	0.274 ± 0.010[b]	0.031
total putrefactive SCFA[2]	1.20 ± 0.088	1.25 ± 0.175	0.940 ± 0.043	0.089
total SCFA	30.9 ± 1.464	29.1 ± 2.824	27.9 ± 1.172	0.324
SCFA profile [μmol/100 μmol total SCFA]				
acetic	71.3 ± 0.893	69.6 ± 2.977	72.3 ± 1.079	0.348
propionic	13.0 ± 0.741	13.3 ± 0.964	12.8 ± 0.906	0.678
butyric	11.9 ± 0.567	12.4 ± 2.167	11.5 ± 0.616	0.651
SCFA pool [μmol/kg BW]	1,123 ± 70.70[a]	868 ± 124.9	767 ± 44.46[b]	0.013
Ascending colon				
SCFA concentrations [μmol/g digesta]				
acetic	16.0 ± 0.649[b]	15.8 ± 0.830[b]	18.8 ± 0.590[a]	0.010
propionic	2.73 ± 0.222	2.65 ± 0.247	3.00 ± 0.161	0.284
iso-butyric	0.248 ± 0.054	0.273 ± 0.040	0.260 ± 0.026	0.696
butyric	2.29 ± 0.163	2.56 ± 0.209	2.51 ± 0.194	0.366
iso-valeric	0.233 ± 0.023	0.238 ± 0.038	0.184 ± 0.014	0.196
valeric	0.248 ± 0.042	0.212 ± 0.030	0.210 ± 0.022	0.435
total putrefactive SCFA	0.729 ± 0.063	0.723 ± 0.049	0.654 ± 0.021	0.303
total SCFA	21.8 ± 0.832[b]	21.8 ± 0.698[b]	25.0 ± 0.788[a]	0.010
SCFA profile [μmol/100 μmol total SCFA]				
acetic	73.7 ± 1.284	72.5 ± 1.949	75.3 ± 0.781	0.202
propionic	12.5 ± 0.781	12.2 ± 1.159	12.0 ± 0.491	0.732
butyric	10.5 ± 0.627	12.0 ± 1.324	10.0 ± 0.623	0.176

C, control; FM, fish meal; MWM, Mealworm larvae meal. [1]Short-chain fatty acids. [2] Sum of iso-butyric, iso-valeric and valeric acids. [a,b]Mean values within rows with no common superscripts are different at $P<0.05$.

group FM ($P<0.05$ vs. C and MWM).

As shown in Table 7, none of the experimental dietary treatments affected the cecal concentrations of total and major SCFA, i.e. acetic, propionic and butyric acids. An analysis of PSCFA revealed a significant decrease in valeric acid content in the MWM group ($P=0.031$ vs. C and FM) and a statistical tendency towards decreased cecal concentrations of iso-valeric acid and total PSCFA ($P=0.057$ and $P=0.089$, respectively) The cecal SCFA pool, expressed as μmol per kg of body weight, decreased significantly in response to the MWM treatment as compared with group C. In the ascending colon, the concentrations of acetic acid and total SCFAs increased significantly in chinchillas fed the MWM diet (both $P=0.010$ vs. C and FM). The profile of major SCFAs in the cecum and ascending colon was not affected by the dietary treatments.

Discussion

The body weight of chinchillas presented in Table 3 can be compared (with due caution) with the findings of Gasco *et al.* [5] who demonstrated that the addition of insect oil to rabbit diets increased perineal fat deposition in their carcasses. In the present study, higher digestibility coefficients of total protein and crude fat were noted in group MWM (Table 4), which could be associated with the higher body weight of chinchillas in this group, resulting from higher fat deposition. According to Poyraz *et al.* [24], the body weight of chinchillas at pelting time is affected by animal care, housing conditions, birth weight, feeding, feed quality and genetic factors. The body weight of chinchillas noted in the present study are higher than those reported by Lanszki [19] and Poyraz *et al.* [24], which points to the high genetic quality of animals and adequate nutrition standards on the farm where the experiment was performed. In an experiment conducted by Głogowski *et al.* [7], daily feed consumption was somewhat higher than in this study, ranging from 21.35 g to 23.04 g, but the experimental animals were younger and rapidly growing. The basic productivity parameters of chinchillas (Table 3), determined in addition to nutrient digestibility coefficients and the parameters of gastrointestinal function, are indicative of good housing and management conditions and appropriate feeding regimes during the experiment.

The coefficients of nutrient digestibility, determined in this study (Table 4), are comparable with those reported by other authors [7, 17, 25], although studies of the type are scarce. Rogier [25] demonstrated that total protein digestibility ranged from 62% to 73% in chinchillas, depending on their age and dietary protein levels. Głogowski *et al.* [7] found that dietary fat levels had no effect on total protein digestibility, which ranged from 69.49% to 71.18% and was somewhat higher than in this experiment. It is worth noting that in rats fed diets supplemented with dried *Tenebrio molitor* larvae, the inclusion of DL-methionine significantly increased the digestibility coefficient of protein from 75.1% to 78.9% [8].

In the present experiment and in a study by Głogowski *et al.* [7], crude fat digestibility was higher in chinchillas fed diets with higher fat content.

It would be difficult to compare the digestibility coefficients of NDF and ADF determined in this study with the findings of other authors because no published data are available for the chinchilla. According to Sakaguchi [26], crude fiber is digested more efficiently by chinchillas and guinea pigs than by rabbits and rats. In a study by Głogowski *et al.* [7], the digestibility of crude fiber ranged from approximately 33% to 38%.

Marono *et al.* [21] analyzed the total protein digestibility of *Tenebrio molitor* and *Hermetia illucens* larvae meals under *in vitro* conditions, with the use of an enzymatic method. The cited authors found that crude protein digestibility was affected by chitin levels in the meals. In both insect meal samples, total protein digestibility was negatively correlated with the content of ADF and chitin. However, average chitin yields in mealworm larvae were estimated at 4.92% of dry matter [16, 28]. Therefore, the effect of chitin in this study could not be significant due to its relative low content of the MWM diet.

The use of fishmeal as a supplemental protein source in chinchilla diets led to a considerable increase in the pH of gastric digesta, compared with both other groups. Further research is needed to confirm or reject the above observation since the activation of pepsin and absorption of selected nutrients rely on the acidic pH in the stomach. Gastric juice, whose main component is HCl, plays an important role in protecting the gut from pathogens. A prolonged increase in gastric pH induced by disease or other factors may promote bacterial overgrowth in the stomach [20]. Unlike the MWM treatment, the FM treatment increased the relative weights of small intestinal tissue (statistical tendency) and digesta (significantly). The MWM treatment decreased viscosity and the FM treatment increased dry matter concentration in the small intestine. An increase in the amount of small intestinal

digesta in group FM chinchillas could be due to its decelerated transit and/or hindered nutrient transport across the intestinal wall (cf. the lower digestibility of total protein and fat in group FM), which could be linked with increased digesta viscosity as compared with the MWM treatment. Greater bulk of intestinal digesta may contribute to increasing the weight of the intestinal wall. Such a physiological effect was observed by Krupa-Kozak *et al.* [18] in a study on rats, which revealed that mucosal volume was positively correlated with digesta mass, concluding that physical stretching of the small intestinal epithelium was the most important causal factor. The decrease in the activities of mucosal sucrase, maltase and lactase, observed in the MWM treatment, could be associated with the higher lignin (ADL) content of mealworm larvae meal as and the MWM diet. Lignin is a phenolic high molecular weight biopolymer which could be responsible for the reduced activity of selected endogenous and bacterial enzymes [15, 27].

Similarly to rabbits, chinchillas are true non-ruminant herbivores with a specific digestion pattern referred to as large bowel fermentation [29, 31]. The chinchilla's cecum and colon are well-developed intestinal segments where less digestible nutrients are efficiently utilized by microbial populations. Gut microbiota utilize the products of fermentation of dietary fiber and, to a lesser extent, of dietary and endogenous proteins to produce SCFA which have emerged as important signaling molecules with diverse physiological effects [22]. The present experiment revealed that fermentation processes in the cecum were most intense in group C chinchillas whose diet contained more SBM compared with FM and MWM treatments. The above observation was confirmed by the highest SCFA pool. Other authors, who compared dietary treatments with different amounts and types of fibrous components, reported that the SCFA pool was a more reliable indicator of the rate of large gut fermentation than SCFA concentration [35]. Interestingly, the supplemental dietary protein sources, i.e. fishmeal and mealworm larvae meal, induced different responses of the large gut in chinchillas. The FM treatment contributed to a significant increase in cecal ammonia concentration, enhanced the extracellular and intracellular activities of bacterial α- and β-glucosidase, α- and β-galactosidase, β-xylosidase, and the intracellular activity of α-arabinopyranosidase in the cecum, relative to the MWM treatment. Unlike the FM treatment, the MWM group was characterized by a significantly lower cecal SCFA pool than the control group. In chinchillas fed the FM diet, the increased ammonia concentration in the cecal digesta could result from the increased amount of dietary protein that escapes digestion in the

upper gastrointestinal tract and enters the cecum. Decreased total protein digestibility was noted in the FM treatment (statistical tendency) vs. groups C and MWM. The decrease in dietary protein digestibility could be responsible for elevated cecal concentrations of PSCFA in the FM group, in particular the concentrations of isovaleric and valeric acids. The increased concentration of cecal ammonia observed in the FM group did not contribute to an undesirable rise in the pH of digesta. The acidity of cecal contents is determined by various factors, including SCFA and ammonia concentrations as well as the buffering capacity of intestinal digesta. De Blas *et al.* [4] reported that buffering capacity and SCFA concentrations are variables of paramount importance, whereas ammonia concentration is only slight positively related to cecal pH in rabbits.

Colonic fermentation processes in chinchillas from control and FM groups were similar as indicated by comparable weights of ascending colonic tissue and digesta, digesta pH, SCFA concentrations and profile. In group MWM, the fermentation site shifted from the cecum to the colon, which was reflected in an increase in SCFA concentrations, greater bulk of digesta and a statistical tendency towards lower colonic pH. Such effects should be considered as adaptation mechanisms of microbial population in the large intestine aimed to derive more energy from less digestible dietary components (e.g. ADF and ADL fractions) that escape digestion in the small intestine and microbial fermentation in the cecum. As a result, the digestibility of dietary gross energy was comparable in all animals.

The results of this study indicate that chinchilla diets can be supplemented with small amounts of animal protein such as fish meal and dried mealworm larvae meal without compromising nutrient digestibility. Crude fat digestibility was highest in chinchillas fed the MWM diet. Both alternative protein sources incorporated into experimental diets improved the digestibility of ADF and ADL. A considerable increase in the activity of cecal intracellular and extracellular bacterial enzymes was noted in the FM treatment, which however did not increase the concentrations of SCFAs. The inclusion of mealworm larvae meal in chinchilla diets had a beneficial influence on crude fat digestibility and shifted the bacterial fermentation site from the cecum to the colon, thus enabling to derive additional energy from less digestible dietary components.

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