ORIGINAL ARTICLE

https://doi.org/10.21708/avb.2023.17.2.11353

Hematological changes in dogs fed with *Tenebrio molitor* larvae meal

Alterações hematológicas em cães alimentados com farinha de larva de Tenebrio molitor

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ABSTRACT: The aim of this study was to evaluate the haematological and biochemical parameters of dogs submitted to different levels of inclusion of mealworm meal (0, 2.5, 5 and 7.5%) in their diet. Four adult females aged 5 years, castrated, with an average weight of 15.8 kg were used. A Latin square design was used, with 4 treatments and 4 replications. The base diets were calculated based on the NRC and provided in the proportion of 80% dry food and 20% wet food. The animals were dewormed, clinically evaluated and adapted to the base diet for 10 days prior to the insertion of me. After fitting, blood was collected for evaluation of haematological and biochemical parameters. The experimental period of each treatment was 14 days, with a new blood collection on the 15th day, with the animals in the fasted state. The blood parameters evaluated were blood count and biochemical tests, composed of urea, creatinine, alanine aminotransferase, alkaline phosphatase, cholesterol, total proteins and their fractions, glucose, triglycerides, C-reactive protein, fibrinogen and immunoglobulin E. The data obtained were subjected to multiple analysis of variance at the 5% significance level. The results showed that none of the mealworm protein inclusion levels showed blood alterations. Therefore, it can be concluded that the use of mealworm meal did not harm the health of the animals tested in experiment, showing that it can be an alternative protein source and can be safely included up to a level of 7.5% in dog foods.

KEYWORDS: Pets; protein; sustainability; mealworm.

RESUMO: O estudo objetivou-se avaliar os parâmetros hematológicos e bioquímicos de cães submetidos à diferentes níveis de inclusão da farinha da larva de *Tenebrio molitor* (0%, 2,5%, 5% e 7,5%) à sua dieta. Foram utilizadas 4 fêmeas adultas com 5 anos, castradas, peso médio de 15,8kg. Utilizou-se o delineamento em quadrado latino, com 4 tratamentos e 4 repetições. As dietas bases foram calculadas com base no NRC e fornecidas na proporção de 80% de alimento seco e 20% de alimento úmido. Os animais foram desverminados, avaliados clinicamente e adaptados à dieta base por 10 dias que antecederam a inserção da farinha dde Tenebrio. Após a adaptação coletou-se sangue para a avaliação dos parâmetros hematológicos e bioquímicos. O período experimental de cada tratamento foi de 14 dias, havendo nova coleta sanguínea no décimo quinto dia, com os animais em jejum. Os parâmetros sanguíneos avaliados foram hemograma, e exames bioquímicos, compostos por ureia, creatinina, alanina aminotransferase, fosfatase alcalina, colesterol, proteínas totais e suas frações, glicose, triglicérides, proteína C reativa, fibrinogênio e imunoglobulina E. Os dados obtidos foram submetidos à análise de variância múltipla a 5% de significância. Os resultados demonstraram que, em nenhum dos níveis de inclusão da proteína de tenébrio houveram alterações sanguíneas. Portanto, concluir-se que o uso da farinha de *Tenebrio molitor* não apresentou prejuizos a saude dos animais testados neste experimento, evidenciando que pode ser uma fonte proteica alternativa e ser seguramente incluída até o nível de 7,5% em alimentos para cães.

PALAVRAS-CHAVE: Animais de estimação; proteína; sustentabilidade; *Tenebrio molitor*.

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Received: 07/05/2022. Accepted: 04/27/2023

INTRODUCTION

The rampant multispecies population increase, both human and animal, raises concerns about sustainability, since the food industry for both shares the same nutrient sources, generating food competition. According to Swanson et. al. (2013), nutritional sustainability is the ability of a food system to feed and provide safe and adequate nutrition, maintaining the health of the population without compromising the ability of future generations to meet their nutritional needs.

As a result, the search for additional new protein sources for animal and human food has intensified, especially for carnivores such as dogs and cats, which share some amino acid requirements and, consequently, protein sources (Boland et al., 2013). In this context, insects have been proposed as an alternative and high-quality protein source, as, in addition to contributing to its sustainability, they can be raised on various sources of organic waste (Van Huis et al., 2013).

Insects have great potential for several reasons: (i) their nutritional value, (ii) their feed conversion efficiency, (iii) the small space required for cultivation and (iv) because they are almost omnivorous, they can grow on diverse substrates (Pinotti et al., 2019; Berggren et al., 2019; Bajuk et al., 2021). Insect proteins have nutritional advantages in terms of their total protein content and/or essential amino acid profile over plant proteins, e.g. beans, cereals, soy or lentils (Bajuk et al., 2021). They may also have advantages over animal meat due to their high content of high-quality protein (~50%–85%) and significant amounts of other nutrients such as minerals, vitamins, and lipids, including omega-3 and omega-6 fatty acids in favourable proportions. Proteins derived from edible insects also have high digestibility (up to 75%–98%) compared to other protein sources (Sun-Waterhouse et al., 2016; Bajuk et al., 2021).

According to FAO (2013), insects have a high protein potential, efficiency in food conversion, in addition to growing and reproducing quickly. Some studies have demonstrated the viability of producing insects and using them as an alternative sustainable protein in the animal diet of poultry, pigs, cattle and aquatic animals (Veldkamp et al., 2012).

Thus, the aim of this study was to evaluate the effect of including mealworm (*Tenebrio molitor*) larvae meal at four different levels (0, 2.5, 5 and 7.5%) in the extruded diet of adult dogs on blood parameters (haematological and biochemical).

MATERIAL AND METHODS

Ethical approval was obtained from the Ethics in Animal Experimentation Chamber (CEUA) with protocol nº 237/2019.

The animals were housed in semi-covered kennels with a smooth cement floor and walls measuring 2.5 x 5 x 1.60 m (width x depth x height, respectively) and with feeders and drinkers in adequate quantities. During the day, the animals were released into a leisure area, consisting partially of a cement floor and partially of earth, to exercise and interact with the other animals, and separated at feeding time.

Four adult female dogs, of mixed breed, with an average body weight of 15.80 ± 4 kg, and approximately 6 years of age, were used. The experimental design used was a Latin square, consisting of four treatments with different levels of inclusion of mealworm meal (0, 2.5%, 5% and 7.5%) in the diet and four replications per treatment. The results were analysed with ANOVA using the R® software with the help of the Candisc package (differences were considered statistically significant at $p \leq 0.05$).

The base diets were calculated based on the NRC (2006) and provided in the proportion of 80% dry food and 20% wet food, formulated based on the animals' body weight. Where animals used about 33% of their protein needs for metabolic maintenance and 67% of their protein needs for growth.

Mealworm meal was incorporated as a substitute for the reference ration. This diet consisted of two commercial products, a dry food and a wet food, in the ratio of 80:20, respectively, as shown in Table 1.

Table 1. Proportions (% and grams) of dry, wet and insect meal fed to the dogs, individually, in their respective experimental period.

Animals		Period 1	Period 2	Period 3	Period 4
1	dry ration	80% (164 g)	77.5% (158.88 g)	72.5% (148.62 g)	75% (153.76 g)
	wet ration	20% (41 g)	20% (41 g)	20% (41 g)	20% (41 g)
	Meal	0%	2.5% (5.12 g)	7.5% (15.38 g)	5% (10.26 g)
2	dry ration	75% (206.6 g)	80% (220.36 g)	77.5% (213.48 g)	72.5% (199.7 g)
	wet ration	20% (55 g)	20% (55 g)	20% (55 g)	20% (55 g)
	Meal	5% (13.76 g)	0%	2.5% (6.88 g)	7.5% (20.66 g)
3	dry ration	77.5% (249.56 g)	72.5% (234.62 g)	75% (241.6 g)	80% (257.6 g)
	wet ration	20% (64.4 g)	20% (64.4 g)	20% (64.4 g)	20% (64.4 g)
	Meal	2.5% (8 g)	7.5% (24.16 g)	5% (16 g)	0%
4	dry ration	72.5% (256.66 g)	75% (265.5 g)	80% (283.2 g)	77.5% (274.36 g)
	wet ration	20% (70.8 g)	20% (70.8 g)	20% (70.8 g)	20% (70.8 g)
	Meal	7.5% (26.56 g)	5% (17.7 g)	0%	2.5% (8.85 g)

To ensure palatability and proper homogenization, mealworm meal was incorporated into the wet fraction. The warranty levels and basic composition of these products, according to the manufacturer, are described in Table 2.

The basic composition of food

Basic Composition (A): Viscera meal, meat and bone meal, chicken fat, wheat bran, ground whole corn, corn gluten meal-21, corn germ meal, soy meal, prebiotic additive (mannan oligosaccharides) (Min. 0.05%), Yucca Extract (Min. 0.04%), Liquid Flavouring Additive (Hydrolysate) of Chicken Guts, Sodium Chloride (Common Salt), Calcium Propionate, Ferrous Sulphate, Copper Sulphate Pentahydrate, Sulphate manganese oxide, zinc oxide, cobalt sulphate, calcium iodate, sodium selenite, vitamin A, vitamin D3, vitamin E, vitamin K3, vitamin B1, vitamin B2, vitamin B6, vitamin B12, folic acid, nicotinic acid, pantothenic acid, biotin, flavouring additive (garlic flavouring), antioxidant additives (B.H.T./B.H.A).

Basic composition (B): Beef offal, pork offal, poultry meat and offal, water, yeast extract, corn cream, vegetable oil, common salt, citric acid, carrageenan/xanana thickeners, garlic, sodium phosphate, sucrose, sodium nitrate, butylated toluene hydroxide (BHT), folic acid, pantothenic acid, biotin, choline chloride, calcium iodate, niacin, pyridoxine, riboflavin, sodium selenite, cobalt sulphate, copper sulphate, manganese sulphate, zinc sulphate, ferrous sulphate, thiamine, vitamin A, vitamin B12, vitamin D3, vitamin E, vitamin K and natural dye haemoglobin.

The mealworm meal used in this study had 45.9% crude protein, 95.9% dry matter, 2.6% Ash and 30.4% ether extract. The animals were dewormed, clinically evaluated and submitted to serological examination for canine visceral leishmaniasis before the beginning of the experiment. In addition, during the 10 days prior to the beginning of the experiment, the animals underwent a period of adaptation to the base diet, consisting of dry and wet food (pate). After the initial period of adaptation, the experimental treatments began with the addition of insect meal, provided twice a day, at 10:00 am and 5:00 pm, for 15 days (experimental period).

After each experimental period, a complete blood count was performed via the automated counting method using flow cytometry (AlereBio-2900 Vet) and differential counting of the leukocyte series using optical microscopy (Nikon E100). Biochemical tests were performed using the Mindray device (BIO-200Vet), and each parameter was analysed using their respective methods: urea (UV enzymatic method), creatinine, total proteins and fractions (colorimetric method), alanine aminotransferase, alkaline phosphatase and cholesterol using the enzymatic method, glucose and triglycerides (colorimetric enzyme), C-reactive protein (latex agglutination), fibrinogen (coagulometric-Clauss) and immunoglobulin E (chemiluminescence).

RESULTS AND DISCUSSIONS

The results of the experiment are described in Table 3, demonstrating that, using the ANOVA test ($P > 0.05$), the levels of inclusion of meal in the diet did not result in significant changes in the blood parameters of the animals in relation to the control group.

Based on the 0% treatment, which refers to the control group, it can be seen that there was no significant difference between treatments based on the analysis of whole blood such as erythrocytes (erythrocytes), platelets and elements of the white series (total leukocytes, neutrophils, eosinophils and lymphocytes), as well as for biochemical analysis, such as total proteins and their fractions, albumin and globulin, Alanine Aminotransferase (ALT), alkaline phosphatase (AP), urea and creatinine. All values remained within those established as normal for adult dogs, as well as triglycerides, total cholesterol, blood glucose and fibrinogen. The variables C-reactive protein and immunoglobulin E were absent in this treatment according to the value and/or characteristic established by the reference.

Melo et al. (2006) described that the percentage of protein in the diet altered some haematological variables, such as the erythrogram, without harming the organic defence system, but this was not observed with the diet in the present study, where the values of erythrocytes remained within the values established as normal for dogs, in agreement with the results of the study by Lisenko et al. (2023) and Nap et al. (1991),

Table 2. Guaranteed levels in dry food (Commercial Product A) and wet food (Commercial Product B), used as a control diet for dogs receiving *Tenebrio molitor* meal at different inclusion levels (0, 2.5%, 5% and 7.5%).

Item	Commercial Product A	Commercial Product B
Humidity (105°)	100 g/kg	105 g/kg
Crude Protein (minimum)	206 g/kg	93.4 g/kg
Ether Extract (minimum)	55.6 g/kg	49.8 g/kg
Crude fibre (maximum)	43 g/kg	13 g/kg
Ash (maximum)	70.9 g/kg	40 g/kg
Total Digestible Nutrients (TDN)	779 g/kg	770 g/kg

Table 3. Means, standard deviation, coefficients of variation and P values for each blood component in their respective treatments (0, 2.5, 5 and 7.5%).

Blood Parameters	0%		2.5%		5%		7.5%		P value
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Albumin	3.05	0.60	2.93	0.55	3.12	0.22	2.94	0.50	0.96
Cholesterol	171.75	24.20	241.70	30.58	196.68	13.34	228.24	39.92	0.14
Creatinine	0.97	0.06	0.90	0.14	1.00	0.10	0.93	0.09	0.65
Eosinophils	13.50	23.38	19.50	33.77	57.00	59.89	13.00	22.52	0.62
Erythrocytes	7.01	0.71	6.41	0.30	6.63	0.06	6.98	0.38	0.30
AP	15.80	3.23	39.10	17.12	20.00	8.85	25.60	18.84	0.23
Fibrinogen	200.00	0	200.00	0	175.00	43.30	175.00	43.30	0.54
Glucose	84.48	3.60	91.72	5.22	89.59	11.94	78.24	0.21	0.69
Globulin	4.28	0.82	4.29	0.55	3.76	0.79	3.76	0.90	0.72
Immunoglobulin E	.	.	.	.	.	.	.	.	.
Leukocytes	5775.0	426.5	6000.0	1238.9	6275.0	1112.2	5425.0	506.8	0.85
Lymphocytes	1701.0	195.4	2039.0	157.7	2163.0	484.7	1480.0	136.0	0.11
Neutrophils	4001.0	358.8	3843.5	1130.2	3951.5	756.2	3821.8	482.7	0.85
Platelets	377750	155477.3	241750	63993.7	368500	108594.4	371250	56108.7	0.36
C-reactive protein	.	.	.	.	.	.	.	.	.
Total Proteins	7.33	0.79	7.23	0.50	6.88	0.62	6.70	0.47	0.57
ALT	50.00	5.87	52.00	7.14	66.00	36.68	91.50	22.42	0.13
Triglycerides	100.65	31.21	106.56	15.08	84.28	7.17	95.19	28.90	0.36
Urea	18.63	1.13	20.34	4.23	20.52	2.95	24.96	3.36	0.14

*There was no significant difference by the ANOVA test at the 5% level of significance (P > 0.05); standard error of the mean (SEM); Alanine Aminotransferase (ALT); alkaline phosphatase (AP); Coefficient of Variation (CV).

who also evaluated blood parameters after feeding diets containing different levels of mealworm protein (7.5% and 15%) and crude protein from feed (14.6%, 23.1% and 31.6%), respectively. Neither author observed differences (P > 0.05) between red blood cells and haematocrit values.

Given the results shown, it appears that mealworm meal did not affect any of the physiological functions of the dogs, corroborating the results obtained by Tubin et al. (2020), who offered mealworm meal up to a level of 20% for tilapia and demonstrated that up to a level of 10%, platelets and leukocytes did not change (P > 0.05), nor did the differential leukocyte count (lymphocytes, neutrophils, monocytes, eosinophils and basophils).

When variations in acute-phase proteins, which undergo alterations in the face of the inflammatory response, such as albumin, fibrinogen and C-reactive protein, were analysed, no variation was observed (P > 0.05), and these proteins remained within the reference values (Nakamura et al., 2008; Cray et al., 2009). Therefore, the inclusion of mealworm meal presents itself in a positive way for use in canine diets, without alteration of acute-phase proteins.

In the study by Andrade et al. (2019), using healthy dogs submitted to diets with three levels of crude protein (28.82% and 31.75%), they did not observe variation in the values of total serum proteins. Safadi et al. (2021), in an experiment, noted that the highest protein values were in obese dogs, and these values could not be attributed to the diet, since the reason for the difference was the elevation of globulin and not of albumin, as is seen in diseases such as obesity, which was not the case in this study.

The analysis of the renal markers urea and creatinine showed no alterations as the protein level was altered. Carneiro et al. (2011), evaluating the haematological parameters, renal and hepatic function of Great Dane dogs in growth superfed with a diet rich in protein (34%), expected to find high levels of serum urea in dogs fed with a high percentage of protein. However, there was no difference (P > 0.05) for the mean serum levels of urea; these values were within the reference intervals (Bush, 1999).

By contrast, Santos et al. (2008), when evaluating the liver function of spayed bitches submitted to a weight gain program, observed a higher mean urea concentration for the animals, associating this result with the high consumption of protein. In contrast to what happened in the present study, where when the protein in the diet was increased (from 0% to 7.5% of protein), the values of urea and creatinine were similar

(according to the mean and standard deviation) and remained within of the reference value. These results are similar to those observed by Lisenko et al. (2023), in their study with mealworm meal at 7.5% and 15% included in the diet of dogs.

As for the enzymes, despite higher mean values of PA in the group that received 7.5% and ALT in the group that received 5% in the present study, there was no statistical difference ($P > 0.05$), and even when they were increased, they remained within the established reference range. Safadi et al. (2021) made the same observation, where the enzymes tended to have higher mean values in the group of obese dogs than in the ideal weight group, but within the reference range for the species.

Triglycerides and cholesterol showed no significant difference ($P > 0.05$) at different levels of ingested protein, remaining within the reference range. In the work by Safadi et al. (2021), with obese dogs, although an increase in cholesterol and triglycerides was expected, this was not verified. Such data corroborate the values found in the study by Kilburn et al. (2020), who measured cholesterol and triglycerides in healthy adult dogs submitted to diets with different levels of Cricket (Gryllodes sigillatus) as a protein source, noting that their levels remained within normal parameters.

As for serum glucose, the dogs showed similar values regardless of the level of protein consumed, not representing a significant difference and remaining within the reference value. Kilburn et al. (2020) evaluating increasing levels (0, 8, 16 and 24%) of cricket meal (Gryllodes sigillatus) in the diet of healthy adult dogs (Beagles), also observed that the inclusion levels of insect meal did not influence serum glucose levels, which were within the desired reference ranges, indicating healthy dogs.

The ingestion of mealworm meal by the dogs did not trigger the production of IgE, cells related to the allergic response, which were then considered absent in blood tests. In view of this, it is noted that the inclusion of such a protein source at the levels studied here is not capable of triggering an allergic reaction. Still regarding immunity, Bovera et al. (2015), in a study with broilers in the growing phase, reported that the blood biochemical values suggested better resistance to diseases and a stronger immune response in the birds, probably due to the prebiotic effects of the chitin present in the diet with the inclusion of insects.

CONCLUSIONS

In general, the inclusion of insect meal in the diet of adult dogs resulted in findings compatible with those found in dogs fed the control diet, indicating that its use as a protein source for dogs is safe, according to the analysed variables and the feeding period provided. It is concluded that mealworm meal, at the inclusion levels and in the experimental period evaluated, can be added to food for adult dogs up to a level of 7.5% without affecting blood parameters and serum biochemicals.

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