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ORIGINAL ARTICLE

Nutritional evaluation and palatability of pet biscuits for dogs

Avaliação nutricional e palatabilidade de biscoitos pet para cães

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Abstract

The study's aim was to evaluate the microbiological, nutritional, textural quality and palatability of pet biscuits made with the inclusion of three flours. The treatments were chicken viscera flour (CVF), commercial Nile tilapia flour (FF) and mixed fish flours of several species (FVF). For the FVF, only the carcasses (backbones) of Nile tilapia, salmon, tuna and sardines were used, and they were washed, whereas FVF as well as the others were cooked for 60 minutes, then pressed, ground and dehydrated for 24 hours at 60° C. After this period, they were crushed and ground for inclusion in the biscuits. The CVF, FF and FVF flours showed 43.82%, 50.48% and 56.70% of crude protein, respectively. CVF had the highest lipid content (10.01%) and FVF had the highest ash content (23.94%) and FVF had the highest caloric value (338.63 kcal 100 g^{-1}). The inclusion of flours in biscuits did not influence the protein and carbohydrate contents, whose average values were 18.48% and 50.23%. However, they influenced the moisture content, lipids, ash and caloric value. Biscuits with the inclusion of viscera (CVF) had the highest lipid content (5.91%) and those with fish had the highest ash content (5.66% and 5.81%) for FF and FVF. Biscuits with FVF had a lower caloric value (318.47 kcal 100 g^{-1}). The microbiological analysis indicated that the biscuits were considered of good quality to feed dogs. Regarding texture and palatability, there was no significant difference between treatments. The inclusion of fish flour, both FF and FVF, is a viable alternative.

Keywords: Calories; Food technology; Nile Tilapia; Tuna.

Resumo

Objetivou-se avaliar a qualidade microbiológica e nutricional, a textura e a palatabilidade de biscoitos pet elaborados com a inclusão de três farinhas. Os tratamentos foram farinha de vísceras de frango (FVC), farinha de tilápia comercial (FF) e mix de farinhas de peixes de várias espécies (FVF). Para FVF, foram utilizadas apenas as carcaças (espinhaço) de tilápia,

salmão, atum e sardinha, as quais foram lavadas. Em todos os tratamentos, as farinhas foram cozidas por 60 minutos, depois prensadas, moídas e desidratadas por 24 horas a 60 °C. Decorrido esse período, as farinhas foram trituradas e moídas para inclusão nos biscoitos. As farinhas FVC, FF e FVF apresentaram 43,82%, 50,48% e 56,70% de proteína bruta, respectivamente. A FVC apresentou maior teor lipídico (10,01%) e a FVF, maior teor de cinzas (23,94%) e o maior valor calórico (338,63 kcal 100 g^{-1}). A inclusão das farinhas nos biscoitos não influenciou nos teores de proteína e carboidratos, cujos valores médios foram de 18,48% e 50,23%, respectivamente. Entretanto, influenciaram no teor de umidade, lipídios, cinzas e valor calórico. Os biscoitos com inclusão de vísceras (CVF) apresentaram os maiores teores de lipídeos (5,91%) e aqueles com inclusão pescado maiores teores de cinzas (5,66% e 5,81%) para FF e FVF. Os biscoitos com FVF apresentaram menor valor calórico (318,47 kcal 100 g^{-1}). A análise microbiológica indicou que os biscoitos apresentaram qualidades apropriadas para serem fornecidos a cães. Em relação a textura e aceitabilidade, não houve diferença significativa entre os tratamentos. A inclusão de farinhas de peixe, tanto FF quanto FVF, é uma alternativa viável.

Palavras-chave: Atum; Calorias; Tecnologia de alimentos; Tilápia do Nilo.

Highlights

- Biscuits chicken and fish viscera flour can be included in preparing PET biscuits for dogs
- Including fish meals in the preparation of pet biscuits for dogs is feasible

1 Introduction

The pet market has been undergoing great development, growth and investment. Thus, with the purpose of attracting investors, research has been developed every year, making the pet market an attractive option for business opportunities (Mazon & Moura, 2017). In Brazil, the population is increasingly appreciating and investing in their animals, for example with toys, baths and grooming and snacks (Matiucci et al., 2020, 2021).

According to the ABINPET- Associação Brasileira da Indústria de Produtos para Animais de Estimação (2021), the pet market revenue was 145.8 billion dollars in 2021, which is equivalent to a significant growth of 11% in relation to the previous year. Animal feed accounts for more than half of the industry's revenue. For the preparation of many of the products consumed by dogs and cats, residues are used or considered to be by-products of food production for human consumption, thus contributing to so-called nutritional ecology, in which residues from one stage of the production chain contribute to the food manufacturing in the following steps (Souza et al., 2022b). While for the Normative Instruction N. 30 of the Ministério da Agricultura, Pecuária e Abastecimento, chewable foods are those products intended for feeding dogs and cats, with the purpose of pleasing or rewarding, although the nutritional value is negligible. In this sense, cookies enriched with fiber or another ingredient to improve their nutritional value is a new alternative in this pet market (Coradini et al., 2015).

Studies indicate that when developing a product for dogs and cats, an attempt has been made to add nutritional and functional value, even in these products intended only for pleasure and reward. Pires et al. (2013) evaluated the palatability of treats enriched with soluble fiber (*Plantago psyllium* L. – psyllium-plantain) for dogs and Souza et al. (2022a, 2022b) analyzed the chemical composition, resistance and palatability of the toothpick treat for dogs with Nile tilapia carcass flour, among other studies. According to Souza et al. (2022a, 2022b), Nile tilapia is recognized as the most consumed fish species by Brazilians and its form of consumption is fillet, generating a high percentage of residues during the filleting process, including the head, scales, skins, viscera and backbone with fins, which can lead to environmental pollution, if there is not an appropriate destination for these residues. To minimize this environmental impact, as well as to bring a healthy alternative, it would be relevant to produce fish flour, one of the better alternatives for the reuse of these residues, with differentiated applicability in animal nutrition, especially for pets. When included in the pet industry, it enriches them nutritionally without altering their organoleptic aspects.

Given the assumptions, the study aimed to evaluate microbiological, nutritional, textural quality and palatability of pet biscuits made with the inclusion of three flours from the reuse of residues from the animal industry.

2 Material and methods

For the production of biscuits for dogs, a formulation was used, differing only in the inclusion of different flours of animal origin. The treatments used were biscuit made with chicken viscera flour (CVF), biscuit with flour from commercial Nile tilapia fillet (*Oreochromis niloticus*) (FF) and biscuit with a mix of fish flour of several species, Nile tilapia, commercial salmon (*Salmo salar*), tuna (*Thunnus thynnus*) and canned sardines (*Sardinella aurita*) (FVF). The flours used in the treatments were purchased commercially, except for those that made up the mix of flours from different species of fish, which were prepared at the Laboratório de Tecnologia do Pescado, at Fazenda Experimental de Iguatemi, belonging to Universidade Estadual de Maringá (UEM).

Tilapia and salmon carcasses without heads and fins were used, as well as tuna carcasses without fins and tails of canned sardines. The skeleton with attached meat remains was considered a carcass. All raw materials came from processing residues from fish processing companies in Rolândia municipality, in the Paraná state (SmartFish Company) (Tomita & Tomita) and Itajaí municipality, in the Santa Catarina state (GDC Alimentos SA). All these residues for the production of the flour mix were transported frozen from their origins to the aforementioned laboratory at UEM.

The raw materials were washed in chlorinated water and weighed and placed separately in industrial pressure cookers (with a capacity of 20 L), containing water, 0.5 mg kg^{-1} of BHT and 0.1 mg kg^{-1} of peroxitane1512®. The residues were cooked in an industrial stove for 60 °C minutes. Afterward, the cooked raw material was pressed in a hydraulic press (capacity of 10 tons). The cake resulting from the press was ground in an industrial meat grinder, placed on aluminum trays and subjected to dehydration in a forced ventilation drying oven for 24 h at 60 °C. After being dehydrated, the masses were crushed and then ground in a knife-type mill, resulting in the fish flour that was used to compose the mixture used in this experiment. The fish flours were packed in plastic bags and stored in a freezer at -18 °C until the experiment was carried out. Chemical and microbiological composition analyzes of fish flours and biscuits were carried out with the inclusion of fish flours, as well as texture and palatability analyses.

2.1 Biscuits preparation

The biscuits were made in a pet food factory located in Maringá city, in the Paraná state. The ingredients used in the formula are summarized in Table 1. During the biscuits manufacture, the ingredients of each treatment were added to an industrial electric mixer and mixed for 15 minutes. After this period, the dough was transferred to the factory's production line, where the dough passed through a cylinder and then the biscuits were cut, separated, baked and packaged for analysis (Figures 1).

Table 1. Pet biscuit formulation with the inclusion of different animal flours.

Ingredients	Content (%)
Wheat flour	62.5
Viscera flour (FVC), commercial fish (FF) and fish mix (FVF)	10.25
Crackling flour	8.56
Sugar	4.38
Premix	0.43
Cookie scrap	1.25
Calcium propionate	1.19
Sodium hexametaphosphate	0.15
Antioxidant	0.03
Emulsifier	0.25
Sodium metabisulphite	0.02

Table 1. Continued…

Ingredients	Content (%)
Water	30.38
Chicken hydrolyzate	2.13
Chicken oil	5.63
Sodium bicarbonate	0.25

Figure 1. Pet biscuit production line. (A) Cutting the biscuits. (B) Separation of the cut biscuits from the dough cylinder, passing through the conveyor belt. (C) Biscuits on the baking mat and the residue returning to be rolled again. (D) Cut biscuits passing through the conveyor belt. (E) Baked biscuits coming out of the oven. (F) Ready-made biscuits.

2.2 Chemical composition in the flours and biscuits

Chemical composition analyzes (moisture and ash) were performed according to the methodology of the Association of Official Analytical Chemists (Ramos et al., 2021). Crude protein contents were determined by the semi-micro Kjeldahl method described by Geiger et al. (1987). For the extraction of total lipids, the method Bligh & Dyer (1959) was used.

Carbohydrate contents were estimated using a mathematical formula that considers the sum of the moisture values, protein, lipids and ash, substituted by 100% (Matiucci et al., 2020). The caloric value (CV) was obtained by the multiplication sum of the crude protein (CP), total lipids (LT) and carbohydrates (CB) content, multiplied by the factors 4, 9 and 4, respectively, according to the formula expressed in Equation 1.

$$CV = (Kcal/100g) = PB \times 4 + LP \times 9 + CB \times 4 \quad (1)$$

2.3 Microbiological analysis and strength test

The microbiological analyzes of the flours and biscuits were carried out for the most probable number (MPN) of Coliforms at 35 and 45° C, the counts of *Staphylococcus coagulase positive* in CFU gram-1 and *Salmonella spp.*, according to APHA (American Public Health Association, 1992).

The microbiological protocol followed the standards recommended by Resolution RDC no. 12, of January 2, 2001, of the National Health Surveillance Agency (Brasil, 2001). To determine the pet biscuits' texture, they were placed on supports with a spacing of two centimeters and were left without support in the Central region. The force was applied in the central region, always at the same point on all biscuits.

The determination of the biscuits' texture parameters was performed using the Brookfield CT3 Texture Analyzer texturometer, using a 50 kg load cell, with a cylindrical acrylic probe with a diameter of 38.1 mm (TA3/100), having as operational parameters: pre-test speed: 2.0 mm s^{-1}; test speed = 0.5 mm s^{-1}; post-test speed = 2.0 mm s^{-1}; force = 50 g; count cycle = 5 seconds; distance = 3.0 mm (Santos et al., 2021). The values represented the arithmetic average of 20 burst force determinations in central slices arranged horizontally on the platform for samples from the same assay (He et al., 2020).

2.4 Pet biscuit palatability analysis

The biscuit palatability test was carried out with a panel of 30 adult dogs, male and female, of different breeds and sizes, belonging to different pet owners. For each animal, there were two challenges. In each challenge, two feeders were shown simultaneously, each containing samples of biscuits to be evaluated (A or B), with preference being given to the one that was totally consumed first or, in cases where there was no total consumption, the one with the highest consumption within 10 minutes. The feeders' position was inverted between each challenge, in order to avoid laterality. These biscuits were offered according to the dogs' weight (Table 2).

Table 2. Pet biscuit units to be offered to dogs depending on their body weight.

Dog weight (kg)	Quantity (units)
1.0 - 5.0	1
5.1 - 10.0	2
10.1 - 15.0	3
15.1 - 20.0	4
20.0 - 30.0	5
30.1 - 40.0	6

The palatability assessment was performed by the intake ratio (RI), according to the formula in Equation 2.

$$RI = food\ A\ intake / \left(food\ A\ intake + food\ B\ intake \right) \quad (2)$$

According to the criteria established by Murakami et al. (2018), due to the small number of repetitions, in this case, RI results are considered significant only above 0.8 of A in relation to B.

2.5 Experimental design and statistical analysis

A completely randomized design was carried out with 3 treatments and 10 repetitions for the analysis of the chemical composition and resistance test of the pet biscuits, with the biscuit being the experimental unit. For palatability analysis or preference test, 30 dogs were used, with three tests per dog.

The software used to perform the statistical analyzes was SAS Studio, version 3.7 (Wicklin, 2008). Statistics were applied to the parameters of chemical composition, physical evaluation or resistance test (shear force) and palatability. For microbiological analysis, no statistics were performed and the analysis was only performed to characterize the animal quality flour used and the product ready to be offered to the animal.

3 Results and discussion

The flours microbiological analysis used in pet biscuits preparation, as well as the ready-made pet biscuits, showed quality, respectively for making and supplying to dogs, depending on the results obtained within the values established by RDC no. 12, from the National Health Surveillance Agency of the Ministry of Health

(Brasil, 2001). For Coliforms at 35 and 45 °C it was <3 NMP g^{-1}, *Staphylococcus coagulase positive* was <102 and absent at 25 g of flour and pet biscuit samples for the analysis of *Salmonella spp.* (Table 3).

Table 3. Microbiological analysis of different animal origin flours used for the preparation of pet biscuits.

Treatments	Microbiological analysis			
	Coliforms at 35°C (NMP g^{-1})	Coliforms at 45°C (NMP g^{-1})	*Staphylococcus coagulase* positive (UFC g^{-1})	*Salmonella spp.* 25g
Flours used in the formulation of biscuits				
CVF*	<3	<3	$<10^2$	Absent
FF**	<3	<3	$<10^2$	Absent
FVF***	<3	<3	$<10^2$	Absent
Biscuits made with the inclusion of different flours				
FVC*	<3	<3	$<10^2$	Absent
FF**	<3	<3	$<10^2$	Absent
FVF***	<3	<3	$<10^2$	Absent

MLN= Most likely numbers. CFU= colony forming unit. *CVF= biscuit made with chicken viscera flour. **FF= biscuit with flour from commercial Nile tilapia fillet (*Oreochromis niloticus*). ***FVF= biscuit with a mix of fish flour of several species, Nile tilapia (*Oreochromis niloticus*), commercial salmon (*Salmo salar*), tuna (*Thunnus thynnus*) and canned sardines (*Sardinella aurita*).

These results showed that the flour used and the pet biscuits were in good condition and regarding the biscuits handling, there was no contamination that prevented their supply to the animals. For the evaluated flours, there was a significant difference in all nutrients and caloric values (Table 4). For the moisture content of the different flours prepared, the one with the lowest content was commercial fish flour FF (12.26%) and the highest was the mix of several species FVF (16.07%) (Table 4). This is due to processing related to the type of raw material, particle size (product milling), as well as the time and temperature for the roasting and dehydration process. However, the final moisture content is an important characteristic, because if this content is high, it contributes to the development of microorganisms.

According to Akhtar et al. (2008), moisture greater than 10% could facilitate bacterial contamination, in addition to the development of mold, while very low moisture could indicate the burning of the ingredient during the flour preparation process.

Table 4. Chemical composition of three flours of animal origin, used in the preparation of pet biscuits.

Chemical composition (%)	CVF*	FF**	FVF***	CV (%)	P value
Moisture	13.47^b	12.26^c	16.07^a	1.58	0.0009
Crude protein	56.70^a	43.82^c	50.48^b	0.42	0.0000
Lipids	10.01^a	9.79^b	6.65^c	3.18	0.0020
Ash	20.37^a	15.32^c	23.94^a	2.47	0.0009
Caloric value (kcal 100 g^{-1})	314.67^a	338.63^a	273.23^c	0.90	0.0003

Different letters on the same line differ by Tukey's test at 5% probability, P value= Significance level, CV= coefficient of variation. *CVF= biscuit made with chicken viscera flour. **FF= biscuit with flour from commercial Nile tilapia fillet (*Oreochromis niloticus*). ***FVF= biscuit with a mix of fish flour of several species, Nile tilapia (*Oreochromis niloticus*), commercial salmon (*Salmo salar*), tuna (*Thunnus thynnus*) and canned sardines (*Sardinella aurita*).

The CVF had significantly higher crude protein content (56.70%) as well as FF (43.82%). According to Silva et al. (2020), viscera flour is the product resulting from the cooking, pressing and grinding of poultry viscera, being allowed the inclusion of heads and feet. It must not contain penalties, except those that may occur unintentionally. Inclusion of all parts resulting from slaughter is permitted, although it must not have hatchery residues and eggshell contamination. The inclusion of these parts and other foreign materials constitutes tampering. The protein in the poultry flour should range from 55% to 65%. Therefore, the viscera flour (CVF) used was within the recommended standard for application in the product, depending on the level of crude protein in the current study.

FVF had significantly the lowest lipid content (6.65%), while CVF had the highest lipid content (10.01%). However, for ash, the flour with the lowest content was FF (15.32%) and the highest ash content was FVF (23.94%) (Table 4). This higher ash content in the FVF was expected, because that this flour was made only with the carcasses (backbone) and a small amount of meat remaining on the bones from the filleting process was present in these carcasses. While FF, fish viscera are also present and often the amount of whole fish was outside the standard for human consumption. The fact that the CVF flour had 20.37% of ash is probably due to the amount of bones present together with the viscera at the time of flour production. According to Silva et al. (2020), the inclusion of heads and feet is allowed, and the product is often used after passing through the pulping machine, and in this process, a small amount of bones can pass.

The caloric value FVF (273.23 kcal 100 g^{-1}) was significantly lower compared to FF (338.63 kcal 100 g^{-1}) and also with CVF (314.67 kcal 100 g^{-1}). This lower caloric value of FVF is due to the low-fat content in the raw material, and it can be seen in Table 4 that FVF had a lower lipid content.

Despite the CVF has a higher protein content when the pet biscuits were made, there was no influence on the protein content of these biscuits. Therefore, for crude protein and carbohydrates, there was no significant difference between the analyzed biscuits, whose averages were 18.43% and 50.23%, respectively for protein and carbohydrates in biscuits of the three treatments, CVF, FF and FVF (Table 5).

There was a significant difference for the levels of moisture, lipids, ash and caloric value (Table 5). Moisture was significantly higher (21.21%) and had lower ash content (4.93%) in biscuits made with CVF, while there was no difference between the biscuits with FF inclusion and the biscuit with FVF, for these parameters evaluated (Table 5). The variation in biscuits' moisture may be related to the flour type used, as seen in Table 4, that is, this is visible from the results shown. Also, it can be inferred that the higher moisture content may due to the higher lipid content present in CVF biscuit, making it difficult for these biscuits to lose moisture (Table 5). Meantime, other factors may still contribute to this high moisture. One of them would passage through the tunnel to bake, mainly because of the position in which they were on this route, because when they passed through the mat, as seen with the naked eye, that is, one of the ends in the longitudinal direction of the mat's length, the biscuits showed a more intense, darker as if they were burning.

Table 5. Chemical composition of three types of pet biscuits with the inclusion of animal origin flours.

Chemical composition (%)	CVF*	FF**	FVF***	CV (%)	P value
Moisture	21.21^{a}	20.29^{b}	20.23^{b}	0.99	0.0287
Crude protein	18.32^{a}	18.24^{a}	18.89^{a}	1.55	0.1919
Lipids	5.91^{a}	5.29^{b}	4.53^{c}	1.78	0.0015
Ash	4.93^{b}	5.66^{a}	5.81^{a}	1.22	0.0018
Carbohydrates	49.63^{a}	50.52^{a}	50.54^{a}	0.84	0.1880
Caloric value (Kcal 100g^{-1})	324.99^{a}	322.64^{a}	318.47^{b}	0.23	0.0071

Different letters on the same line differ by Tukey's test at 5% probability, P value= Significance level, CV= coefficient of variation. *CVF= biscuit made with chicken viscera flour. **FF= biscuit with flour from commercial Nile tilapia fillet (*Oreochromis niloticus*). ***FVF= biscuit with a mix of fish flour of several species, Nile tilapia (*Oreochromis niloticus*), commercial salmon (*Salmo salar*), tuna (*Thunnus thynnus*) and canned sardines (*Sardinella aurita*).

The higher moisture content may also be related to the raw material used to make the biscuit dough. It can be observed during the preparation of the biscuits that even whether the amount of water used is the same for all treatments, the dough of pet biscuits with fish flour was more difficult to shape into a round shape (homogeneously kneaded). Also, according to Cappelli et al. (2020) the speed of the mixer and its force to "knead" are important factors for obtaining a quality biscuits, interfering with the product's final result.

Melini et al. (2017) reported that pet biscuits made with carob and cotyledon flour had 10.7 to 13.3 g 100 g^{-1} crude protein. Furthermore, the authors mentioned pet biscuits enriched with 5% fish flour had a crude protein content of 10.10 g 100 g^{-1}. These values are lower than those found in the pet biscuits of this current study, whose average crude protein value was 18.43%. However, in these studies, the inclusion was 10.25% of animal flour.

Considering that fish proteins are of high biological value due to their adequate profile of essential amino acids, particularly lysine and methionine (Bakare et al., 2020), their incorporation into biscuits can complement cereal proteins normally used in the formulation of cereals biscuits and increase the product biological value.

It can be observed that the lipid content (5.91%) of these biscuits made with viscera flour was significantly higher than the others. The lipid content of these biscuits reflected the lipids results in respective flours, that is, the biscuit with fish flour mix (FVF) (4.53%) and this same flour (6.65%) had lower lipid content, while the biscuits with viscera flour (5.91%) and viscera flour (10.01%) had a higher lipid content (Tables 4 and 5).

Among the biscuits made in the current study, FVF had a significantly lower caloric value (318.47 kcal 100 g^{-1}) and there was no difference between the other two biscuits prepared. Therefore, there is a need to study the fatty acid profile in pet biscuits with the inclusion of these different flours, mainly associated with the omega 3 series fatty acids, as they are present in marine fish, since in mix using FVF it may contain percentages of salmon, tuna and sardines in the formulation. Pet biscuits did not show statistical differences regarding the analysis of the biscuit resistance variables, as shown in Table 6.

Table 6. Resistance analysis of three pet biscuits with the inclusion of animal flour.

Resistance analysis	CVF*	FF**	FVF***	CV (%)
Hardness (g)	4446.5[a]	4908.0[a]	4809.75[a]	0.2342
Deformation in hardness (mm)	0.7120[a]	0.6370[a]	0.6695[a]	0.4021
Hardness effort (mJ)	17.2750[a]	17.6850[a]	17.2750[a]	0.8988
Reference to load resistance (g)	2. 7500[a]	2.0000[a]	4.7500[a]	0.2989
Reference to deformation under load (mm)	2.9545[a]	2.9510[a]	2.9470[a]	0.5995
Fracturability (g)	4446.50[a]	4876.25[a]	4702.50[a]	0.3544

Different letters on the same line differ by Tukey's test at 5% probability, P value= Significance level, CV= coefficient of variation. *CVF= biscuit made with chicken viscera flour. **FF= biscuit with flour from commercial Nile tilapia fillet (*Oreochromis niloticus*). ***FVF= biscuit with a mix of fish flour of several species, Nile tilapia (*Oreochromis niloticus*), commercial salmon (*Salmo salar*), tuna (*Thunnus thynnus*) and canned sardines (*Sardinella aurita*).

Souza et al. (2022a) evaluated the resistance of stick-type pet snacks with the inclusion of different levels of carcass flour and Nile tilapia head residues. The authors reported that there was a decrease in shear force, as the level of inclusion of the flour increased, thus disrupting the very snack collagen structure since its base is made with ox shavings. The biscuit shape and thickness are important parameters for evaluating the product, depending on the results obtained in terms of appearance and its texture or crunchiness. Therefore, it is necessary to evaluate the biscuit hardness parameters. Srivastava et al. (2014) analyzed the parameters of hardness, cohesiveness, elasticity and chewiness using the Texture Expert software, and found a correlation between the product's crude protein content and its texture. However, Pauly et al. (2013) stated that the crude protein content had a greater influence on the biscuits' hardness. According to these authors, the higher the crude protein content is, the greater the hardness of the biscuit will be. Therefore, dough with more protein content can greatly change the geometry and biscuit texture profile (Bakare et al., 2020).

Based on the premises shown above, the biscuits in the current study showed 18.43% of crude protein and an average texture value (fractur ability and resistance) 4675.08 g, corresponding to 45.863 N (due to the multiplication by 9.81 which is the conversion value from kg to N) (Table 6). Meanwhile, Herpandi et al. (2011), making biscuits with residues of *Euthynnus lineatus*, found crude protein content from 8.81 to 14.25%, and the hardness of these biscuits ranged from 35.30 to 78.34 N, with the protein content being lower than those obtained in the current study, with biscuit with the inclusion of FF and FVF (fish flour) and CVF (poultry viscera). Therefore, for the texture of these pet biscuits, there was no statistical difference between the treatments, demonstrating that the inclusion of flours regardless of animal origin (poultry or fish) does not change the characteristics of pet biscuits.

The pet biscuits evaluated with the inclusion of animal origin flour showed no statistical difference for the palatability test regardless of the raw material used as a protein source. Therefore, flours from CVF, FF or FVF are an alternative for dog biscuits, as shown in Tables 7, 8 and 9.

Table 7. Dogs' palatability: Test A – FF (1) vs FVF (2).

Tests	Morning	Afternoon	Total period
FF (1)	11	9	20
Period (%)	55.00	45.00	100.00
Treatment (%)	40.74	33.33	37.04
FVF (1)	16	18	34
Period (%)	47.06	52.94	100.00
Treatment (%)	59.26	66.67	62.96
Total	27	27	54
Total treatment	100.00	100.00	100.00
P - value	0.573	0.601	0.540

Table 8. Dogs' palatability: Test B – FF (1) *vs* FVC (3).

Tests	Morning	Afternoon	Total period
FF (1)	8	11	19
Period (%)	42.11	57.89	100.00
Treatment (%)	27.59	37.93	32.76
CVF (3)	21	18	39
Period (%)	53.85	46.15	100.00
Treatment (%)	72.41	62.07	67.24
Total	29	29	58
Total treatment	100.00	100.00	100.00
P – value	0.401	0.433	0.467

Table 9. Dogs' palatability: Test C – FVF (2) *vs* FVC (3).

Test	Morning	Afternoon	Total period
FVF (2)	11	17	28
Period (%)	39.29	60.71	100
Treatment (%)	37.93	58.62	48.28
FVC (3)	18	12	30
Period (%)	60.00	40.00	100.00
Treatment (%)	62.07	41.38	51.72
Total	29	29	58
Total treatment	100.00	100.00	100.00
P - value	0.114	0.131	0.220

Hall et al. (2018) developed a palatability study with small, medium and large dogs and observed that diets containing higher levels of crude protein had better palatability results, both in terms of consumption and preference. Knight & Satchell (2021) also stated that high levels of crude protein in dog diets interfere with palatability, digestibility and faeces quality. In addition to the high crude protein content, some authors mention that high fat content also contributes to palatability results, as a preference for moist or semi-moist foods (Meineri et al., 2021; Samant et al., 2021).

Souza et al. (2022b) in their study tested different levels of carcass flour inclusion with Nile tilapia head residues. These ingredients were included in a toothpick treat for dogs. The authors reported that there was a decreasing preference for dogs as the level of inclusion of fish flour in these treats increased. With the inclusion of fish flour, according to these authors, there was a reduction in the snacks' protein content. Probably the inclusion of fish flour has contributed to the reduced pet snack palatability. Therefore, it was a very different result from the one observed in the current study, with the pet biscuits with the inclusion of fish flour and poultry viscera, the authors did not find any difference in the crude protein content, nor in the palatability between the biscuits, being able to be included any of the flours analyzed in the current study.

4 Conclusions

Concerning the inclusion of fish meal, both commercial Nile tilapia flour and fish's flour mix - backbone of Nile tilapia, salmon, tuna and canned sardines flour are viable alternatives for the preparation of dog biscuits.

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