REBRAPA
Brazilian Journal of Food Research

Brazilian Journal of Food Research
ISSN: 2448-3184

https://periodicos.utfpr.edu.br/rebrapa

Valorization of industrial byproducts, okara and beer bagasse, to design balanced laboratory animal feed

Sofia Marchioni
marchioniperez@gmail.com
http://orcid.org/0009-0005-1706-454X
Facultad de Química, Bioquímica y Farmacia, Instituto de Investigaciones en Tecnología Química (INTEQUI), Universidad Nacional de San Luis, San Luis, Argentina

Mercedes Edith Campderrós
mcampd@gmail.com
http://orcid.org/0000-0002-4286-0485
Facultad de Química, Bioquímica y Farmacia, Instituto de Investigaciones en Tecnología Química (INTEQUI), Universidad Nacional de San Luis, San Luis, Argentina.

Gabriel Emiliano Castro
gabrielemilianocastro@gmail.com
http://orcid.org/0009-0004-1625-0394
Facultad de Química, Bioquímica y Farmacia, Instituto de Investigaciones en Tecnología Química (INTEQUI), Universidad Nacional de San Luis, San Luis, Argentina.

María Victoria Ostermann-Porcel
vic.ostermann@gmail.com
http://orcid.org/0000-0001-7844-2106
Facultad de Química, Bioquímica y Farmacia, Instituto de Investigaciones en Tecnología Química (INTEQUI), Universidad Nacional de San Luis, San Luis, Argentina.

ABSTRACT

The exploitation of industrial byproducts as raw materials was proposed to develop food for laboratory animals. Okara and beer bagasse were primarily considered based on their protein content and abundance in our country. The Pearson square method was used to balance the formulation, which was designed as pellets. The product was physically, chemically, and biologically evaluated on Balb/c mice for 34 days. The results were compared with a control batch fed with a commercial food. The results showed that it was possible to produce pellets with physical characteristics suitable for consumption by experimental mice. The composition of the formulation developed was: 25.54% protein, 19.3% fat; 1.50% fiber; 10.46% ash; 3.51% moisture; 39.69% carbohydrates. Brea gum, a natural product, was employed to achieve an adequate consistency. Mice fed with this product demonstrated good acceptance, palatability, and assimilation. A better feed conversion index and a higher protein efficiency ratio were obtained regarding the commercial food, which would indicate that the food developed had better quality proteins. A lower daily consumption was evidenced due to the fat content of this sample, which provides a higher amount of calories per gram. This work is a contribution to a sustainable production with a positive impact on the environment.

KEY-WORDS: protein-rich feed; circular economy; sustainable feed formulation; brea gum; biological assessment.

INTRODUCTION

The food industry is currently focused on addressing waste management and recycling issues through valorization, which involves using by-products and discarded materials to create new value-added products for commercial use. Waste valorization offers alternatives to disposal or landfilling, enabling the reuse of nutrients in the production of primary products, thereby highlighting its potential benefits (Anal, 2017). Argentina generates various agro-industrial byproducts, such as okara (from soybeans) and beer bagasse (from the brewing industry), which have great potential for food applications. These byproducts are rich in certain nutrients, particularly proteins. Beer malt bagasse is the most abundant byproduct, constituting about 85% of the waste from brewing. For every 100 L of beer produced, 15-20 kg of bagasse is generated (Lopes et al., 2021). Malt barley bagasse, a potential fiber source, offers several dietary benefits when incorporated into human nutrition (de Oliveira Silva et al., 2022). Studies by Ivanova et al. (2017) and Mussatto et al. (2006) have shown that malt bagasse contains vitamins, minerals, and amino acids like valine, alanine, serine, glycine, glutamic acid, and aspartic acid. Okara is the by-product left after extracting the soluble fraction during the production of soy beverages or tofu. It is one of the most significant solid residues from bean crushing, rich in dietary fiber, protein, and phytochemicals like isoflavones, saponins, and minerals, which have potential health benefits. For every kilogram of soybeans, 1-1.8 kg of wet okara is produced, resulting in large quantities generated daily in the industry (Ostermann-Porcel et al., 2016). Despite its potential, the use of okara in feed formulations has been limited due to various nutritional and technical challenges. However, the food industry byproducts hold significant potential for the development of animal feed. (Ajila et al., 2012). Diverse feed formulations have been reported in the literature (Barszcz et al., 2014; Jeyakumar et al., 2009) for laboratory animals. However, no previous studies have been published on the production of pellets using okara and/or beer bagasse as raw materials.

Based on this, the objective of this study was to evaluate okara and beer bagasse as alternative raw materials for the development of an innovative, nutritionally balanced feed by assessing their chemical, nutritional, physical, and textural properties. Additionally, the formulation must exhibit optimal textural characteristics, specifically hardness and resistance, as well as an appealing appearance, to ensure its acceptance by animals without rejection. In this regard, the incorporation of brea gum as a hardening agent further enhances the uniqueness of the formulation. The brea gum is an abundant natural resource sourced from the native forest. This approach aims to promote sustainable production with a positive environmental impact, aligning with the principles of the circular economy. Bioassays were performed to evaluate animal acceptance of the pellet formulation. These tests, using laboratory animals as experimental models, remain the standard methods for assessing the protein quality of feed ingredients.

MATERIAL AND METHODS

RAW MATERIALS

According to the technical standard for animal feed of the Argentine Republic (SENASA, Res. Nº 594/2015, National Service of Agrifood Health and Quality. Technical Standard for Animal Feed of the Argentine Republic.), animal feed is

understood as any product, industrialized or not, that is consumed by the animal, and is capable of contributing to its nutrition, promoting its growth, maintenance, reproduction, productivity, or adaptation to a better state of health.

The raw materials used in the production of the balanced feed (BF) were selected taking into account their physicochemical composition, mainly their protein content: Fish flour (60% proteins) was supplied by Harinas SAO S.R.L. (Rio Negro, Argentina). This product has no food preservatives or additives, is 100% natural, and is made with raw material extracted from the San Matías Gulf. Soybean flour (40% proteins), wheat flour (10% proteins), and maize flour (7% proteins) were purchased in the local market.

The beer bagasse was provided by Kerze craft brewery (San Luis, Argentina). Samples of 95% light and 5% dark malt barley bagasse, a by-product of the production of "Sunset" type beer, were used. The high initial moisture content and the presence of components such as fermentable sugars and proteins make the wet bagasse susceptible to microbial contamination. The moisture content was reduced in the laboratory by pressing, and subsequent drying was carried out in an oven (San Jor, Argentina) for 7 hours at 65±5°C to reduce the microbial load (or to limit the growth of microorganisms) (de Oliveira Silva et al., 2022). After that, the sample was dried in an electric oven (Ultracomb, Argentina) for 3 hours at 65±5 °C to reduce the moisture content into values suitable for converting the bagasse into a flour-like product. Temperatures higher than 45 °C generate enzymatic and non-enzymatic browning reactions, with characteristic derived odor-volatiles. Finally, the dried bagasse (B) was ground using a blender (Peabody, Argentina) with a power of 1000W. The sample was stored in airtight containers and kept at 5±1 °C until used (a_w = 0.35).

Okara flour was prepared in the laboratory based on the methodology described by Ostermann et al. (2017). The soybean was soaked in water for 8–10 h at 22 ± 1°C. The hydrated soybeans were ground using the blender, incorporating small amounts of water at 100 ± 1°C to enhance the grinding. A thermal treatment was applied during 20 min at 90 ± 1°C to reduce the activity of the trypsin inhibitor and deactivate the lipoxygenase enzyme that causes an unpleasant taste (Ostermann Porcel et al., 2016). After that, the suspension was allowed to cool and was filtered through a clean cloth. The filtration process yielded a beige liquid (soymilk) and a granulated solid, the wet okara, which is the by-product of interest in this work. Finally, wet okara was placed in molds and pressed to reduce water content, and then it was placed in a cylindrical container and dehydrated using a microwave oven (BGH, Argentina) for 1 hour with agitation to promote vapor release (for a batch of 400 g of wet okara). The dried okara sample (Ok-D) was subjected to a grinding process to reduce the particle size and stored in an airtight jar (a_w = 0.29).

As additives for the formulation, calcium carbonate (E170, Biopack), sodium bicarbonate (E500ii, Biopack), and sodium chloride (Biopack) were used, which act as preservatives and flavor enhancers. To extend shelf life, calcium propionate (E282, Biopack) was added. All additives used in this study were food grade and are defined in the Argentine Food Code (CAA). Additionally, brea gum provided by the Native Forest Area of the Provincial Ministry of the Environment (San Luis, Argentina) was employed. The native material was purified at the laboratory following the methodology described in Clapassón et al. (2020). The brea gum is approved as a thickening, stabilizing, and emulsifying agent in the Argentine Food Code (Chapter XVIII, Article 72.1).

FORMULATION AND PROCESSING OF ANIMAL FEED PELLETS: STANDARDIZATION OF THE FEED FORMULATION

Formulation development

Balancing a ration consists of combining two or more ingredients in the right proportions and quantities to achieve a balanced diet for the different species and categories. To develop diets that meet the needs of the animals, information on the composition of the raw materials, as well as the nutritional requirements of the species, must be available.

The Pearson Square method is a model for calculating a diet. It consists of a manual calculation method to determine the best combination of ingredients when a limited number of ingredients is considered. Two to "n" ingredients can be used, where "n" is an even number. The essence of this method is to calculate the proportion of food that allows achieving the desired content of the nutrient to be balanced. The condition to be fulfilled is that half of the ingredients to be used must have a higher value than the target parameter to be attained in the formulation (e.g., protein percentage). Using this method, it was possible to calculate the percentage share of each ingredient, and the amount expressed in grams (g) needed to produce a certain amount of feed.

The production of BF starts with the processing of quality raw materials, which is important to ensure a feed that meets the animal's needs. Ok-D, B, and maize flour were subjected to a grinding process with a commercial grinder (Peabody, Argentina) to reduce the particle size of the samples. Then, the samples were manually sieved (mesh size of 200 µm). Grinding is an important step in feed production, as the particle size of the raw materials influences the final quality of the pellets. In addition, a smaller and more homogeneous particle size will prevent size segregation in the mixer, allowing a homogeneous product.

The raw materials and additives were weighed using a precision analytical balance (Kern 770, USA). Then, raw materials were mixed in a planetary mixer (Santini, Argentina) following a specific incorporation sequence and with different agitation times (Fig. 1) to ensure that each portion of the mixture presented all the nutritional parameters for which the BF was designed. When the incorporation of additives was evaluated, they were added after all the solid ingredients had been mixed. Subsequently, the liquids (water 57.2g/100g and corn oil 34.2g/100g) were added, and the dough was kneaded to the desired consistency. The commercial corn oil used had the following composition (per 13 mL serving): total fat 12 g (saturated fat 1.2 g; monounsaturated fats 4.8 g; polyunsaturated fats 6 g), Vitamin E 7.7 mg.

Pellets were produced using the extrusion method. The laboratory-scale system operated on the principle of compressing the processed mass through an extruder screw. This process involved the rotation of a screw, which transported the material to the pellet-forming die with an 11mm diameter (the diameter of the cutting funnel), where it was compacted to attain its final geometry. The particle size of the raw materials directly influenced the level of compaction; smaller particle sizes resulted in reduced interstices between particles, facilitating greater product compaction. For pellet production, an electric meat mincer (Jenny, 415-290, Argentina) was utilized. The process involved changing cutting plates with three different diameters (12.5, 6, and 4 mm) to optimize dough compaction. Subsequently, a manual meat mincer was employed, fitted with a die to achieve the desired pellet diameter and final length through manual cutting. To optimize pellet hardness, various concentrations of

calcium carbonate, sodium chloride, sodium bicarbonate, and brea gum were assessed. However, in this work, we only discuss the combination that yielded the most favorable results concerning the hardness parameter: calcium carbonate (3% w/w), sodium chloride (3% w/w), sodium bicarbonate (1.5% w/w) and brea gum at two different concentrations (10% and 20% w/w).

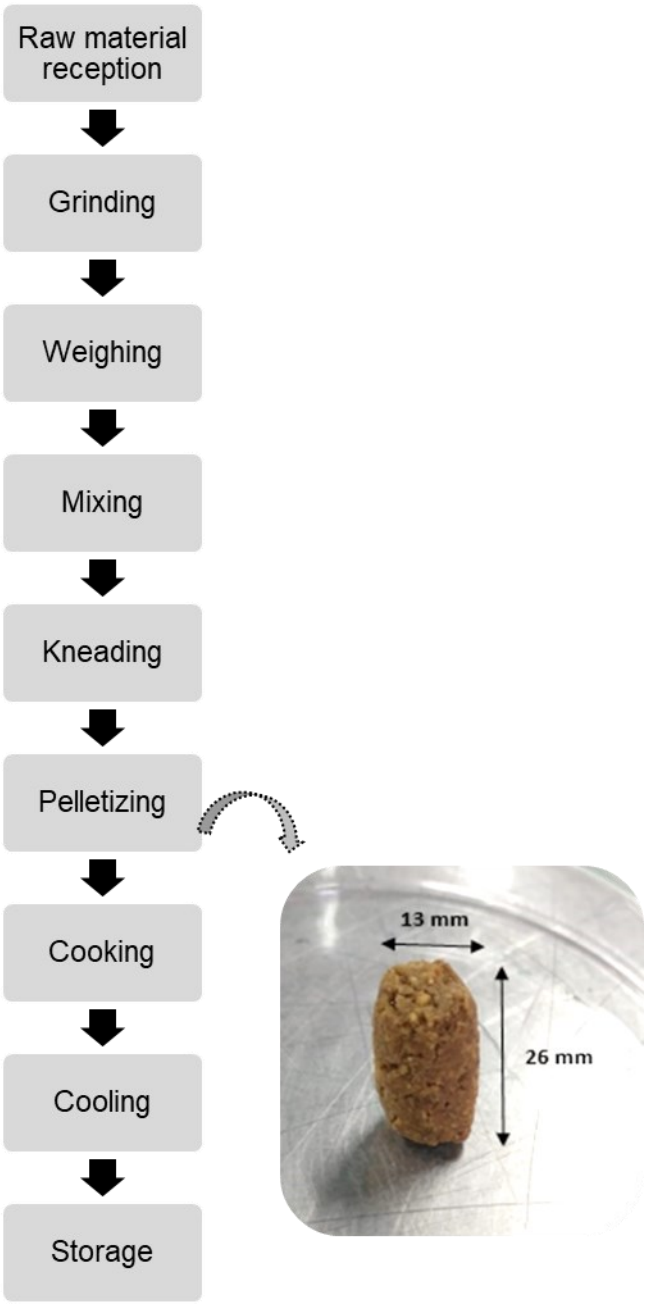

Figure 1. Flowchart of the process of obtaining a balanced feed.

Pellets were dried in an electric oven (Ultracomb, Argentina) at 120 °C for 55 minutes. This temperature ensured bacterial load control and induced the gelatinization of proteins, leading to the breakdown of starches that acted as natural binders. Complete starch breakdown converted these simple starches into sugars, which, upon cooling, bound the ingredients together. The cooling rate was carefully

regulated to prevent potential issues during pellet storage and transport. Samples were cooled for 10 minutes at 4±1 °C, and evaporative cooling was completed at (22 ±1 °C) over 18 hours. The pellets were then weighed, labeled, packaged, and stored under stable conditions to safeguard against chemical, physical, or microbiological contaminants until transportation to the animal facility.

The optimization and standardization of the formulation involved the evaluation of the texture profile (TPA) of the formulated balanced feed pellet, which served as a key parameter for quality assessment.

TPA DETERMINATION

Texture profile analysis for the final product (pellet)

The texturometer (TMS-TOUCH, Food Technology Corporation, USA) was used. The product was supported on two parallel supports, separated by a distance of 13 mm. A third parallel axis with a cylindrical acrylic specimen (diameter 39 mm and length 21 mm) was moved vertically, exerting a compressive force, load cell 500N, until a break in the product structure was produced. Ten samples were analyzed at a displacement speed of 70 mm/min. The hardness parameter was determined from each force-time curve recorded for each sample as the peak point of the linear section of the curve. The mean and standard deviation of these 10 results were reported.

Texture profile analysis for the dough

The test was performed in a back-extrusion container (50 mm in diameter), filled 75% with the sample, using an acrylic cylinder probe (25 mm) attached to an extension bar. A load cell of 500 N was used. Three replications were made at a test speed of 30 mm min^{-1} and a distance of 10 mm. Mean values were used to obtain a force–time curve, calculating the following as texture parameters: (i) firmness = maximum compression force in extrusion thrust into sample (g); (ii) consistency = area within a curve during extrusion thrust (g s); (iii) cohesiveness = maximum compression force during withdrawal of probe from sample (g); (iv) viscosity index = area within negative region of curve during probe withdrawal (g s).

PHYSICAL CHARACTERIZATION

The physical properties of the pellets developed, identified as (PE), were determined and compared with the control sample (CS) in order to obtain a product with similar characteristics. The control sample is a commercial complete balanced food for animals (Asociación de Cooperativas Argentinas (ACA), approved by SENASA, Argentina) which covers by itself the daily nutritional requirements of the animals, of a species, category and given physiological state, for which it is intended, according to what is defined in Annex I 8-28-15 Technical Standard, of SENASA, Argentina (National Service of Agrifood Health and Quality). The following parameters were evaluated:

Physical dimensions of the pellets

The length (mm), diameter (mm) were measured with a right, and the weight (g) per unit and bulk density (g/mL) was determined by filling a volumetric cylinder with a known quantity of pellets. The bulk density was calculated as the mass of the sample per unit volume of the sample (g/L) (Aarseth et al. 2006).

Colorimetric analysis

A Mini Scan EZ digital spectrophotometer (Hunterlab, USA) was used. The colorimeter was calibrated with a black/white color standard (External checking and calibration standards, provided by the supplier). The results reported are the average of three measurements on each sample in the coordinates of the CIELAB color space, which consists of a Cartesian system defined by three color coordinates: L*, a*, and b*. The whiteness index (WI) was calculated according to Equation 1.

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}] \quad \text{(Equation 1)}$$

In addition, the color difference (ΔE) between the samples and the reference was calculated using the following equation (Equation 2).

$$\Delta E = \sqrt{(\Delta L *)^2 + (\Delta a *)^2 + (\Delta b *)^2} \quad \text{(Equation 2)}$$

Where ΔL^*, Δa^*, and Δb^* are the differences between the color coordinates of the sample and those of the reference. This parameter provides a quantitative assessment of the color variation among samples.

CHEMICAL COMPOSITION

The physicochemical characterizations of dried okara (Ok-D) and beer bagasse (B) were carried out, as these two raw materials were processed and conditioned in the laboratory for their subsequent use. Physicochemical analyses were performed in triplicate according to AOAC methods (Association of Official Agricultural Chemists 2016). Carbohydrates were determined by difference.

Chemical composition analysis is necessary in closed formula diets, which are subjected to unpredictable variations due to changes in raw materials or variations in premixing and mixing before pelleting or extrusion. It is also necessary in nutritional studies in which these diets are used as a standard or control. The proximate analysis includes quantitative determinations of the following parameters: proteins, fiber, fat, ash, and moisture. The total caloric value (TCV) was calculated considering the Atwater factors (Food and Drug Administration (FDA) (2001): 4 (calories per gram of protein and carbohydrate) and 9 (calories per gram of fat) and using Equation 3 to calculate the TCV, where CH=carbohydrates; P=Proteins and L=Lipids.

$$TCV = 4(CH + P) + 9L\ [=]\ kcal\ g^{-1} \quad \text{(Equation 3)}$$

Determination of water activity (aw)

The sample was previously grounded, and the a_w was measured using an Aqualab PRE (Argentina).

PRELIMINARY EVALUATION OF THE BIOLOGICAL ASSESSMENT

The Faculty of Chemistry, Biochemistry, and Pharmacy at the National University of San Luis has a facility, known as Biotherium, for the use and care of laboratory animals, ensuring ethics, welfare, and legality in animal experimentation. It currently has strains of Balb/c mice, Knockout mice, and Wistar

rats. These animals are bred under strict sanitary barriers, seeking to maintain their microbiological quality.

Biological methods are based on weight gain or nitrogen retention in tests with experimental animals, which are fed diets containing the protein to be analyzed (FAO/OMS). The animal protocol carried out in this study was reviewed and approved by the Bioethical Committee of the National University of San Luis (Q-388/21, RCD 02 - 67/2022), and measures for the use and care of laboratory animals of the University were followed.

For the preliminary evaluation of the biological assessment of the balanced food elaborated in the laboratory, a factorial experiment was designed with two factors: gender and treatment. Factor 1: Gender; Two levels: Male and Female. Factor 2: Treatment; Two levels: pellets produced in the laboratory (PE) [Treatment 1] and control sample (CS) [Treatment 2], resulting in a total of four groups (n_groups). A power analysis was conducted to determine the minimum sample size using an ANOVA statistic to ascertain whether significant differences exist in the acceptance level of the PE compared to the CS (Bhandari, 2023). The following factors were taken into account: n_groups = 4; Effect size (f) = 2; significance level (α error) = 0.05, Power (1-β) = 0.8. The analysis yielded as result a total sample size of 8 individuals. The actual power achieved was slightly higher than anticipated, reaching 0.82. This indicates the robustness of our experimental design and ability to detect significant differences in the acceptability of the balanced food between genders and treatments.

The quality of the balanced feed was evaluated in a preliminary form by considering not only its chemical and nutritional composition but also by assessing the animal's acceptance of the feed and its ability to digest and utilize these nutrients. Eight Balb/c mice (4 male and 4 female) were obtained from Biotherium. Animals were housed individually in ventilated cages in a temperature-controlled room (20 ± 1°C) with a 12-hour light/dark cycle. Cages are made of AISI 304 quality stainless steel with provision for fixing the water bottle and provision for feeding paddles. Fresh and clean water was available *ad libitum,* and it met the required quality standards. The *in vivo* evaluation of the feed formulations was carried out under the following conditions: a) Feeding hours: Feed is permanently available *ad libitum*, animals eat an amount of food that is determined by their energy requirements (Beynen and Coates 2001), average intake: 2.5 g/day (females) and 5.5 g/day (males). b) Environmental conditions: Temperature 22 ± 1 °C. c) Age: 21 ± 2 days from 45 to 50 g weight. d) Weighing time: 10:00 h. e) Light conditions: light/dark cycle 7:00- 19:00 h.

The mice were divided into two groups, 2 male mice and 2 female mice each. One group was assigned to the new diet (PE) consisting of the pellets produced in the laboratory, while remaining mice were maintained under standard diet as control sample (CS). The groups were respectively called: i) Control Group (CG) and ii) Experimental Group (EG). Both groups of mice were initially fed with a standard diet for one week before the PE diet started. All mice were maintained on their allotted diet for 34 days. The main idea in this stage was the evaluation of acceptability and palatability of the new rodent feed developed from industrial byproducts. Every day in the entire study period, body weight and food consumption were measured and registered. Different parameters were evaluated that allow us to relate feed intake to animal weight gain and protein efficiency ratio (Boyd & McNevin, 2022).

Feed consumption (FC)

Weekly feed consumption was determined for each animal. Feed consumption was determined by weighing the amount of feed provided and subtracting the remainder that may have been left in the trough after 1 week (Equation 4). This value is expressed in grams/week.

FC = weight of feed provided - weight of feed left over (Equation 4)

Weight gain (WG)

WG was calculated using Equation 5, expressed in grams.

WG = Final weight - Initial weight (Equation 5)

Feed conversion index (FCI)

This index (dimensionless) is obtained using Equation 6.

FCI = Feed consumed/WG (Equation 6)

Protein efficiency ratio (PER)

This ratio allows us to evaluate the quality of protein in feed. PER was calculated according to Equation 7 (Silva et al., 2003).

PER = WG (g) / [FC (g) * %Protein] (Equation 7)

STATISTICAL ANALYSIS

A one-way analysis of variance (ANOVA) with Fisher's LSD test ($p<0.05$) to measure central tendency (mean) and standard deviation (SD), with a confidence level of 95%, was used for statistical analysis of results. All analyses were carried out using Statgraphics Centurion XVI software (StatPoint Technologies Inc., Warrenton, USA).

RESULT AND DISCUSSION

RAW MATERIALS

As mentioned in the materials and methods section, all the raw materials used in this study were purchased from the market, except the Ok-D and B, which were prepared in the laboratory. Given that these flours were processed internally, it is essential to conduct a thorough characterization to ensure their quality and composition.

Chemical composition of okara and beer bagasse

In this study, the valorization of okara and beer bagasse was investigated as potential raw materials in the development of a balanced feed suitable for laboratory animals. Drying and handling for storage are important steps to

preserve the quality of dried okara (Ok-D) and beer bagasse (B). The composition of Ok-D and B depends mainly on the dehydration process to which they were subjected, in addition to the residual moisture of the sample and the seeds and grains used to obtain them. This dependence would explain possible variations in the compositions reported by different authors (Martins Muliterno et al., 2017; Mateos-Aparicio et al., 2010; Redondo-Cuenca et al., 2008). Both materials are rich in fiber, low in fat, and are also of vegetal origin, provide carbohydrates, and have a low ash content. The results of the physicochemical analysis of the dried samples are shown in Table 1. Water activity (a_w) is a crucial factor influencing the stability and quality of food products, affecting processes like lipid oxidation, nonenzymatic browning, enzyme activity, and microbial growth (Stępien & Grzyb, 2023). The values obtained for Ok-D (0.29) and B (0.35) suggest that Ok-D is more stable in these aspects, with a lower a_w inhibiting the growth of microorganisms and the occurrence of oxidation reactions. Lower a_w values, typically below 0.6, significantly reduce microbial growth, which is crucial for extending the shelf life of food products (Tapia et al. 2020). Additionally, higher a_w values above 0.6 favor the proliferation of microorganisms, including bacteria and molds, as well as enzymatic reactions, such as those involved in spoilage and browning (FAO, 2003). It is important to highlight that a_w is not only influenced by the water content but also by the composition and structure of the raw materials, which can vary significantly between different food types. Therefore, maintaining low a_w levels is often a strategy to extend shelf life and ensure product safety (FAO, 2003).

It can be observed in Table 1 that the main component of Ok-D is protein (~34.61%). Other authors report that okara is an excellent source of fiber and high-quality protein (Guimarães et al., 2020). The overall nutrient availability of Ok-D depends on several factors, such as the variety of soybeans, the climatic conditions, and the amount of water added and extracted in the obtaining process (Kamble and Rani, 2020). Préstamo et al. (2007) stated that okara has an approximate protein content of 30% with a good essential amino acid profile and in vitro digestibility, making it an economical source of plant-based protein. The fat that remains in Ok-D was found to be 19.1%, which is notably higher than the value typically reported in the literature, where the fat content in okara is generally around 10%. Guimarães et al. (2020), in their study, reported fat values for okara flour obtained through different drying methods, including 9% for forced air oven drying at 70°C, 8.9% for microwave drying, and 10.44% for freeze drying. These values are significantly lower than the fat content found in our okara sample. The discrepancy could be attributed to differences in the processing conditions, as the drying methods used in Guimarães et al.'s study were relatively milder compared to those that may have influenced the higher fat content in our sample, such as the specific drying technique or potential variations in the initial raw material content. Moreover, dried okara contains a high proportion of polyunsaturated fatty acids, particularly linoleic and linolenic acids, which are characteristic components of its lipid profile. These unsaturated fatty acids contribute to the nutritional and functional properties of okara, reinforcing its value as a byproduct for food applications despite the variation in fat content observed. Additionally, small quantities of starch, sugars, potassium, and notable levels of B-group vitamins are also found in okara. Okara offers health benefits due to its antioxidant, hypolipidemic, and hypoglycemic properties (Feng et al., 2021; Dai et al., 2019). Ok-D presented a fiber content of 20.37%; in this sense, Dai et al. (2019) informed that okara has a higher amount of insoluble dietary fiber (92% of the total dietary fiber) than soluble dietary fiber.

Table 1. Results of water activity and physico-chemical characterizations of dried okara (Ok-D) and beer bagasse (B).

Sample	Fat %(w/w)	Moisture %(w/w)	Proteins %(w/w)	Fiber %(w/w)	Ash %(w/w)	Carbohydrates %(w/w)	aw
Ok-D	19.10±4.67	1.38±0.14	34.61±1.49	20.37±1.20	3.44±0.41	21.11	0.29±0.06
B	2.60±0.01	25.56±0.14	15.23±0.32	9.43±2.38	3.06±0.68	44.12	0.35±0.02

NOTE: Results are expressed as mean ± SD

The beer bagasse sample had a protein content (15.23%) higher than the value reported by Rigo et al. (2017), who reported a 12.5% protein content in malt bagasse flour, but lower than the value found by de Oliveira Silva et al. (2022) (18.87 %). As we mentioned, the protein content in malt bagasse is mainly influenced by the type of cereal used in beer processing. Regarding the fat content, a percentage of 2.60% was obtained; it is a value lower than that found by de Oliveira Silva et al. (2022) and Rigo et al. (2017), who obtained 5.52% and 5.9% of lipids in malt bagasse flour, respectively. The essential amino acids present in the malt bagasse represent approximately 30% of the total protein content, with lysine being the most abundant. The rest are non-essential amino acids, including mainly histidine and glutamic acid (Waters et al. 2012). Papageorgiou & Skendi, (2018) established that the processing by-product of barley contains a significantly higher amount of vitamin E compared to the whole barley grain. Moreover, it is a rich source of various bioactive compounds, including phytates, phenolics, and insoluble dietary fiber.

Weiskirchen et al. (2020) stated that grain-based diets containing natural ingredients such as soybean meal, ground corn, fish meal, animal byproducts, and very high levels of both soluble and insoluble fibers can be chosen for their nutritional benefits or to meet certain dietary requirements. From the results obtained, it can be seen that Ok-D and B present nutritional and technological interest in the manufacture of new products in the food industry, as raw materials, since the dried samples obtained in this research have higher protein content than other raw materials commonly used in the production of balanced feed as wheat flour with 5% and maize flour 9% (those ingredients, for instance, are listed in the commercial sample that we used as control sample in this study). Malenica et al. (2023) establish that fruit and vegetable wastes (FVWs) are an excellent source of protein, and those proteins in FVWs are often used as valuable ingredients for the manufacture of feed components for livestock. Some protein content of selected FVWs are tomato pomace (22.21%) and cold-pressed rapeseed cake (33.20%). In addition, diets enriched with different dietary fibers such as barley beta-glycan, apple pectin, inulin, and others have recently been studied (Drew et al., 2018). However, no evidence was found regarding the incorporation of okara and beer bagasse into the same matrix as the balanced feed.

CHARACTERIZATION OF THE BALANCED FEED DEVELOPED

Formulation and processing of animal feed pellets: Standardization of the feed formulation

The balanced feed (BF) was produced in pellet form by agglomerating and compressing the mixture into compact units using a mechanical pressing process

and subsequent application of heat in order to remove the remaining moisture. The calculated formulation, using Pearson's square method, to produce a 500 g batch of balanced feed is detailed in Table 2. Raw materials used in this formulation are ingredients of different diets for both animals and humans (Đukić et al., 2022; de Olivera Silva, 2022).

Table 2. Formulation, using Pearson's square method, to produce a 500 g batch of balanced feed (PE-F3).

Raw Material	%Protein	Amount (g)
Fish Flour	60	136.80
Soybean Flour	37.3	118.56
Okara (Ok-D)	36.1	90.15
Bagasse (B)	15.23	5.02
Maize Flour	9	10.49
Wheat Flour	5	114
Corn Oil/water	-	171/286
Additives		**Amount (g)**
Calcium carbonate		14.25
Sodium chloride		14.25
Sodium bicarbonate		7.12
Brea gum		95
Calcium propionate		0.83

When comparing the hardness values of the pellets, we observed that the BF initially developed in the laboratory (PE-F1=30.05±3.66 N) presented significant differences compared to the control sample (CS=125.78±2.02 N). The hardness values of PE-F1 formulation were not considered suitable for consumption in mice since rodents are prone to malocclusion as their incisors, or long front teeth, have open roots and continue to grow throughout the animal's life (Legendre, 2003). In nature, rodent teeth are naturally worn down through the consumption of hard food and gnawing behavior. To overcome this limitation, different concentrations of hardener additives were evaluated, respecting the limits established by the Argentine Food Code for their use. However, in this work we only mention the combination that gave the best results, regarding the hardness parameter: calcium carbonate (3% w/w), sodium chloride (3% w/w) and sodium bicarbonate (1.5% w/w) as anti-caking agent and acidity regulator. In addition, brea gum at two concentrations (10% and 20% w/w) were evaluated. These formulations were codified PE-F2 and PE-F3 respectively. The latter formulations showed the following hardness value: PE-F2= 45.68 N and PE-F3=100.04 N. The PE-F3 formulation presented the highest hardness value, an adequate value for the consumption of the rodents in the Biotherium. A similar value of pellet hardness was obtained by Oike et al. (2021), who attempted to reduce diet hardness by replacing some nutritional ingredients with 8% dried egg powder. They reported that the hardness of the diet affects the progression of age-related hearing loss.

Molecules as proteins, glucose and fructose, and additives as calcium carbonate can increase molecular interactions such as hydrogen bonds, van der Waals forces, hydrophobic interactions or modify the electrical charges of the medium, improving the affinity between the components of the formulation (Hoyos et al., 2017). In addition, the binders present in a matrix, such as starch, promote the increase of the affinity between the different components of the formulations through changes in the rheological properties of the medium, like the

increase of viscosity when starch, proteins or gums are added (Giraldo et al., 2017). The results of hardness showed that the addition of brea gum significantly improves the hardness parameter in pellets ($p<0.05$). Indeed, brea gum has technological properties similar to other natural gums such as xanthan gum or konjac gum, for which Han et al. (2022) recently reported results as hardeners, improving the texture. For the above-mentioned, the sample PE-F3 was selected as optimal to continue the study.

A back-extrusion test was performed on the mass of the PE-F3 formulation, from this analysis it is possible to obtain values for firmness (σf), consistency, cohesiveness and adhesiveness. The firmness (maximum positive force) was 125.92 N. Cohesiveness ("Strength" of internal bonds maintains the structure of a sample) represents the resistance of a material to a second deformation in relation to how it behaved in a first deformation cycle. For the produced balanced feed, a value of 2.90± 0.35 N was obtained. The consistency (maximum negative force of the curve) was 46.36 N and the viscosity index (negative area under the curve) presented a value of 1.09 N.s.

Physical Characterization

Once the pellet hardness was optimized, physical parameters of the sample were evaluated (Table 3). The PE-F3 pellets presented dimension values, bulk density and pellet weight without statistically significant differences compared to the control sample (CS).

Table 3. Physical characterization of CS and PE-F3 samples.

Sample	Length (mm)	Diameter (mm)	Bulk density (g/mL)	Pellet weight (g)	L*	a*	b*	WI
CS	23.5±0.85^{a}	14.9±0.74^{a}	0.39±0.26^{a}	2.03±0.07^{a}	44.59±0,15^{b}	7.47±0.09^{b}	25.18±0.05^{b}	38.68
PE-F3	25.2±0.91^{b}	13.8±1.13^{a}	0.41±0.22^{a}	2.11±0.13^{a}	27.62±0.71^{a}	5.51±0.16^{a}	19.79 ±0.76^{a}	24.76

NOTE: Within each column averages followed by the same letter are not significantly different ($p>0.05$). Results are expressed as mean±SD.

Due to the colorimetric diversity of the raw materials used in the formulation of the BF, a colorimetric analysis of the processed pellets (PE-F3) was required, as well as the comparison with the CS. Table 3 shows that sample PE-F3 had a lower whiteness index (WI) than the CS. This accounts for the incorporation of Ok-D and B, which have a lightness (L*) of 65.59 and 53.13 respectively. During the drying process to which these samples were subjected, reactions occur that lead to the darkening of the samples, such as the Maillard reactions between reducing sugars and proteins. With regard to the colorimetric parameters a* and b*, it was found that the feed obtained had a less reddish (lower a*) and less yellowish (lower b*) coloring, compared to CS. When conducting the colorimetric analysis, statistically significant differences were observed in the parameters (L*, a* and b*) between the PE-F3 and CS samples ($p<0.05$). The color difference analysis between the CS and PE-F3 samples revealed a ΔE value of 17.91, which correlates with a very noticeable color difference to the naked eye. This variation could be due to significant differences in the composition of the samples or variations in the

manufacturing process. The lower L* value in the PE-F3 sample suggests it is darker than CS, while the reductions in a* and b* values indicate a shift towards less reddish and yellowish tones, respectively. These changes in the color space coordinates reflect notable alterations in the visual properties of the samples, which may have implications for sensory perception and the final product's acceptance. However, this variable did not show any restriction regarding food intake.

Chemical characterization

The BF produced seeks to provide the balance of nutrients needed by the animal, which is a challenge to produce a high-quality and low-cost product. Proximate analysis based on the AOAC analytical standard has been performed on animal feed pellets, and the results are shown in Table 4. Water activity (a_w) measurement tests were carried out to determine the quality and safety of the food.

Table 4. Chemical composition and total caloric value (TCV) of CS and PE-F3 samples.

Sample	a_w	Moisture %(w/w)	Fat %(w/w)	Proteins %(w/w)	Fiber %(w/w)	Ash %(w/w)	Carbohydrates %(w/w)	TCV (Kcal g^{-1})
CS (*)	0.41±0.02[a]	12	6	23	6	10	43	318
PE-F3	0.45±0.01[b]	3.51±0.04	19.3±0.04	25.54±0.28	1.50±0.28	10.46±0.08	39.69	422.62

NOTE: Within each column, averages followed by the same letter are not significantly different (p>0.05). Results are expressed as mean±SD.
(*) The chemical composition of CS was provided by the supplier.

Analyzing the values obtained, it can be seen that the moisture content of PE-F3 does not exceed the 10% limit set by the National Research Council Committee (1995) for the formulation of commercial diets for experimental animals. This moisture content guarantees chemical and microbiological stability due to the low water activity (0.41-0.45; Table 4) for bacterial growth, with minimal lipid oxidation, and low rates of non-enzymatic browning (Roudaut, 2020). The manufacturing process, such as drying temperature or process duration, can influence the amount of water removed from the food. If commercial food has undergone a gentler drying process or has gone through an extrusion process that retains more moisture, it could result in higher moisture content compared to laboratory preparation, which may have undergone a more intense drying process.

Proteins are essential components in the dietary requirements of both humans and animals, influencing their growth. They play an essential role in muscle formation and are also components of other molecules. Inadequate intake of essential protein is frequently associated with the onset of diverse diseases in livestock. Consequently, ensuring a sufficient and high-quality protein source is imperative (Malenica et al. 2023). A prominent aspect in the formulation developed is an increase of 11.04% in protein content with respect to the commercial product being one of the main challenges of the work. It is evident that protein plays a significant role in animal diets; however, it is necessary to realize the further assay of digestibility and degradability of protein in PE-F3. Regarding the fat content in the PE-F3 sample is higher than that of the MC sample. However, there is evidence of diets for mice administered with up to 40 % fat without major adverse effects (Cedeño Moblecilla, 2013) and Weiskirchen et al. (2020) stated that

high corn oil dietary intake was shown to improve health and longevity of aging mice when fed at normal energy balance. The drawback in this case would be from the chemical point of view, a high fat content does not contribute to feed preservation due to possible auto-oxidation. In Table 4, we can observe that the formulated balanced food (PE-F3) exhibits a water activity value of 0.4. FAO (2003) mentioned that reaction rates for lipid oxidation decrease as water activity decreases. However, as water activity drops to values below 0.40, the reaction rate begins to increase once again. This generally makes the 0.40 water activity region an important target for the production of, for example, balanced food.

With respect to fiber content, the PE-F3 sample had a lower fiber content than the CS. However, the National Research Council Committee establishes a maximum limit of 10 % of fiber to avoid depressing the efficiency in the consumption performance of other nutrients in the feed. In addition, it provides for a minimum fiber content of 3%, as this would facilitate digestibility in experimental animals. Regarding the role of dietary fibre in monogastric animals' diets, studies have shown the effects of dietary fiber on the health and gut development of non-ruminant young animals. The study concluded that some components of dietary fiber improve gut health and play a role in preventing diarrhea in non-ruminant young animals (Malenica et al., 2023). About the carbohydrates of the developed formulation, they turned out to be slightly lower than the commercial sample.

Finally, the total caloric value (TCV) of the processed feed was calculated and compared with the CS. PE-F3 sample had a higher caloric value, the energy intake is mainly due to the higher fat content of these samples. Herein, PE-F3 represents a hyper caloric diet compared to CS.

Even though agri-food industrial wastes and by-products represent an unconventional highly promising alternative to the current feed materials available in the market, there are certain limitations in the utilization of these wastes and by-products for the development of animal feed that still need to be addressed, including potential decreases in nutrient digestibility, complex safety regulations, and the high costs associated with waste treatment and transportation (Malenica et al., 2023).

Assessment of feeding behavior in laboratory mice

The objective of this study was to determine how diets can impact food intake and appetite. The palatability refers to the physical and chemical properties of the balanced feed that is associated by the organism, generating a need to continue ingesting the feed or to stop consuming it. Biomaterial evaluation is a crucial step in biomedical research, and as part of the preclinical toxicological safety evaluations (Harikrishnan, 2022). Rodents and, in particular rats and mice, are the most widely biomaterials employed for research (Hawkins et al., 2018). Mice were identified and placed in individual housing boxes. All mice were initially fed with a standard diet (CS). Then, CS or PE-F3 diets were furnished to mice, as applicable. To assess the effects of the feeding behavior, the individual growth of the animals (weight gain or weight loss) was evaluated during the experimental period. Growth curves of the two batches of mice were presented in Fig. 2. In the growth curves it can be observed that the mice of the two experimental batches showed an increase in weight over the course of the days. This trend was evident in both gender mice, which would indicate a good acceptance, palatability and assimilation

of the food produced (PE-F3) by the animals. A similar behavior was reported by Gonzalez-Soto et al. (2023), who stated that a moderate-fat diet containing isolated soy protein did not differentially impact body weight gain in male and female mice compared with a diet containing skim milk powder. Male animals fed the PE-F3 diet presented a period of adaptation to the feed, evidenced by a decrease in weight during the first days of consumption generated by a state of diarrhea. In addition, some physical changes were evidenced as frizziness of the coat during the first week of experimentation, attributed to the stress generated by the change of diet. As mentioned in the Materials and Methods section, the week before the assessment, all the animals (both batches) were supplied with commercial balanced feed (CS), ensuring uniform conditions during the adaptation period which continues with research on feed. This pattern was not observed in the experimental females fed with PE-F3. However, it should be mentioned that these animals showed greater hyperactivity than the male mice also fed with PE-F3 during the whole period evaluated. After the first days of adaptation, no significant differences were detected regarding the defecations of the animals, in terms of shape and quantity, in the period studied.

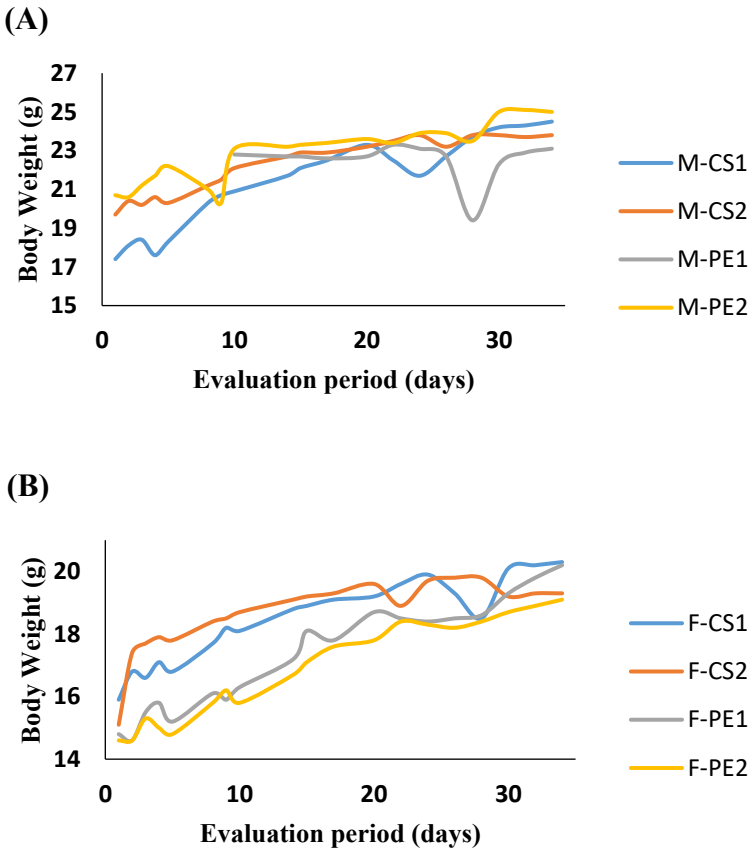

Figure 2. Evaluation of body weight: A) Male mice. B) Female mice.

Finally, different parameters from Eq. 5 to 7 were evaluated, which allowed relating feed consumption to animal weight gain and protein efficiency ratio from the day the animals were weaned until the end of the experiment. Results are presented in Table 5. The result showed that, at the end of the evaluation period, all the experimental animals fed with PE-F3 reached an individual weight similar to that of the animals fed with CS. Once the data on feed consumed was analyzed

regarding to the weight gain obtained during the experiment, it was possible to calculate the Feed Conversion Index (FCI) for each diet supplied, finding that the females fed with PE-F3 made better use of the feed ingested compared to the rest of the animals, which is evidenced by a lower feed conversion ratio. FCI gives an idea of biological efficiency. The efficiency of the animal body to make an efficient use of the food is inversely proportional to the FCI. In other terms, the animal requires less kilograms of feed to gain the same amount of weight. Analyzing the results in Table 5, it can also be shown that the animals fed with PE-F3 diet showed a lower FCI value than the animals fed the commercial diet (CS). Although the results do not show a statistical significant difference ($p<0.05$), these values indicate that PE-F3 is more effective than CS. In order to justify these data, a larger sample population would need to be evaluated. The calculation of protein efficiency ratio (PER) allowed us to obtain an initial approximation of the protein quality of the balanced feed elaborated (PE-F3) compared to the control sample (CS). Hackler (1979) stated that comparisons can be made among the group of diets, and the diet with the highest growth rate will be superior when compared to diets with a lower growth rate. In terms of PER, it can be observed that female mice supplied with PE-F3 diet showed a higher PER than those fed with CS. Considering that PER is a ratio of the weight gained by an animal per unit of protein provided in the food, the results would indicate that the balanced feed elaborated in the laboratory (PE-F3) would contain a better protein quality than CS, and that female mice would exhibit better food assimilation with PE-F3. Conversely, the opposite trend was observed for male mice. However, further study is needed to determine the protein assimilation and digestion capacity by the animals, as well as the determination of metabolic, hormonal, and biochemical profiles.

Even though the biological assessment was started by rationing the same amounts (g) of CS and PE-F3, the control sample was finished 5 days earlier. Table 5 shows that the weekly feed consumption (FC) is higher in the batch fed with the CS. It was decided to replenish CS and continue the evaluation until the balanced feed PE-F3 was finished. It is possible to suggest that a high-fat feed, such as PE-F3, provides a higher amount of calories per gram, which may lead to a reduction in feed intake because the animals meet their caloric needs by ingesting a smaller volume of feed (Warwick & Synowski, 1999).

Table 5. Identification (Id.) of the mice used in the assessment of feeding behavior.

Gender	Food supplied	Id.	W_i (g) (*)	W_f (g) (*)	WG (g)	FC (g/week)	FCI	PER
Male	CS	M-CS1	17.4	24.5	7.1	29	4.085	0.0106
Male	CS	M-CS2	19.7	23.8	4.1	29.1	7.098	0.0061
Male	PE-F3	M-PE1	22.8	23.1	0.3	22.6	7.533	0.0005
Male	PE-F3	M-PE2	20.7	25	4.3	27.4	6.372	0.0061
Female	CS	F-CS1	15.9	20.3	4.4	26.6	6.045	0.0072
Female	CS	F-CS2	15.1	19.3	4.2	27.2	6.476	0.0067
Female	PE-F3	F-PE1	14.8	20.2	5.4	20.2	3.741	0.0105
Female	PE-F3	F-PE2	14.6	19.1	4.5	23.3	5.178	0.0076

NOTE: (*) W_i and W_f represent the initial weight and final weight of animals, respectively.

As mentioned above, the two study animal groups had similar individual weights at the end of the study (W_f). This would indicate that the experimental subjects (M-PE1; M-PE2; F-PE1; and F-PE2) increased food consumption in terms of caloric density and not volume since the hypercaloric diet supplied (PE-F3) contained a higher fat content than the CS. These results suggest that mice tend to regulate their food intake in order to consume the same amounts of calories regardless of changes in diet and the type of food available. Due to this process of food regulation, the experimental subjects maintained a growth curve similar to that of the control subjects. Although the animal evaluation period was short (34 days), it allowed us to verify the acceptability of consumption of the new formulation developed in this study and validate that the weight gain in the batch fed with PE-F3 was consistent with the batch fed with the control sample (commercial).

CONCLUSIONS

The need to reuse industrial byproducts with nutritional value is critical. In this study, two industrial byproducts, okara and beer bagasse, were utilized in the development of a balanced feed for laboratory animals. The results demonstrated that the incorporation of brea gum into the formulation allowed us to achieve the required hardness of the pellets (PE-F3), obtaining physical parameters similar to the control sample (CS). Composition analysis revealed that the formulation elaborated presented higher protein and fat content than the control sample, with low water activity. Biological evaluation carried out in the Biotherium confirmed good acceptance, palatability and assimilation of the balanced feed elaborated for the mice, leading to weight gain with this diet comparable to that to commercial feed during the study period. It is important to highlight that this study was performed on a small-scale using our own design in feed formulation, and further nutritional and digestive research will be needed. Although the results demonstrated the effectiveness of the developed formulation, with good acceptance and assimilation by the animals, some limitations observed in the study should be addressed as potential future work. Nevertheless, it is certain that the gradual understanding of the implementation of by-products for animal nutrition in a circular food system can contribute to future feed production with a lower environmental impact.

Acknowledgments

The authors thank the staff of the Bioterio of the Facultad de Química, Bioquímica y Farmacia, UNSL, especially the veterinarian Jorge Perino for his collaboration in experiments with laboratory animals.

REFERENCES

AARSETH, K. A.; SØRENSEN, M. STOREBAKKEN, T. Effects of red yeast inclusions in diets for salmonids and extrusion temperature on pellet tensile strength: Weibull analysis, **Animal Feed Science Technology**, 126, pp. 75–91, 2006.

AJILA, C.; BRAR, K.; VERMA, M.; TYAGI, R.; GODBOUT, S.; VALÉRO, J. Bio-processing of agro-byproducts to animal feed. **Critical reviews in biotechnology**. 32. 10.3109/07388551.2012.659172. , 2012.

ANAL, A. K. Food Processing By-Products and Their Utilization: Introduction. In **Food Processing By-Products and Their Utilization**; (Ed. Anal AK.) John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2017; pp. 1-10. 2017. https://doi.org/10.1002/9781118432921.ch1

AOAC International. **Official Methods of Analysis of the Association of AOAC International**. 20th Edition, AOAC International, Rockville, MD, 2016.

CÓDIGO ALIMENTARIO ARGENTINO (CAA). Ley 18284/69, Capítulo XVIII (**Food Additives**) Ed. La Roca, Buenos Aires, Argentina, actualized: March, 3, 2021. https://www.argentina.gob.ar/anmat/codigoalimentario

BHANDARI, P. **Statistical Power and Why It Matters - A Simple Introduction**. Scribbr. Available: https://www.scribbr.com/statistics/statistical-power. 2023.

BARSZCZ, M.; TUŚNIO, A.; TACIAK, M.; PARADZIEJ-ŁUKOWICZ, J.; MOLENDA, M.; MORAWSKI, A. Effect of the Composition and Autoclave Sterilization of Diets for Laboratory Animals on Pellet Hardness and Growth Performance of Mice. **Annals of Animal Science**. 14. 10.2478/aoas-2013-0064. 2014

BEYNEN, A. C.; COATES, M.E. In: VAN ZUTPHEN, L. F. M.; BAUMANS, V.; BEYNEN, A. C. editors. **Principles of Laboratory Animal Science. A contribution to the humane use and care of animals and to the quality of experimental results**. Amsterdam: Elsevier, pp 111–127. 2001.

BOYD, C. E.; MCNEVIN, A. Overview of aquaculture feeds: global impacts of ingredient production, manufacturing, and use. **Woodhead Publishing Series in Food Science, Technology and Nutrition, Feed and Feeding Practices in Aquaculture** 2 ed., Woodhead Publishing, pp 3-28, ISBN 9780128215982, https://doi.org/10.1016/B978-0-12-821598-2.00003-5. 2022.

CEDEÑO, M.; EDWIN, L. **Formulación, elaboración y control de calidad de un alimento balanceado para ratones de experimentación (*Mus musculus*) del bioterio de Escuela de Bioquímica y Farmacia de la ESPOCH**. Escuela Superior Politécnica de Chimborazo. Riobamba. http://dspace.espoch.edu.ec/handle/123456789/2691. 2013.

CLAPASSÓN P.; MERINO N. B.; CAMPDERRÓS M. E.; PIRÁN ARCE, M. F; RINALDONI, A. N. Assessment of brea gum as an additive in the development of a gluten-free

bread. **Food Measurement and Characterization**, 14, pp 1665–1670. https://doi.org/10.1007/s11694-020-00414-3. 2020.

DAI, B.; HUANG, S.; DENG, Y. Modified insoluble dietary fibers in okara affect body composition, serum metabolic properties, and fatty acid profiles in mice fed high-fat diets: An NMR investigation. **Food Research International**, 116, pp. 1239-1246, 10.1016/j.foodres.2018.10.011. 2019.

DREW, J. E.; REICHARDT, N.; WILLIAMS, L. M. et al. Dietary fibers inhibit obesity in mice, but host responses in the cecum and liver appear unrelated to fiber-specific changes in cecal bacterial taxonomic composition. **Scientific Report**, 8, 15566. https://doi.org/10.1038/s41598-018-34081-8. 2018.

ĐUKIĆ, N.; RADONJIĆ, A.; POPOVIĆ, B.; KLJAJIĆ, P.; PRAŽIČ-GOLIĆ, M.; GORAN ANDRIĆ, G. The impact of the protein-carbohydrate ratio in animal feed and the initial insect population density on the development of the red flour beetle, Tribolium castaneum. **Journal of Stored Products Research**, v. 97. https://doi.org/10.1016/j.jspr.2022.101983. 2022.

FAO/WHO. Protein Quality Evaluation: Report of the Joint FAO/WHO Expert Consultation, **FAO Food and Nutrition Paper**, 51. Rome: FAO. 1991.

FAO. Handling and Preservation of Fruits and Vegetables by Combined Methods for Rural. Technical manual. In. BARBOSA-CÁNOVAS, G. V.; FERNÁNDEZ-MOLINA, J. J.;

ALZAMORA, S. M.; TAPIA, M. S.; LÓPEZ-MALO, A.; CHANES, J. W. Eds. **General considerations for preservation of fruits and vegetables**. Chapter 3. Rome. ISBN 92-5-104861-4. 2003.

FDA. **Code of Federal Regulations**. Title 21, v. 2, revised as of 1 April 2001. 21CFR101.9. Washington, DC, United States Government Printing Office. 2001.

FENG, Y.; WANG, R.; THAKUR, K.; NI, Z. J.; ZHU, Y. Y.; HU, F.; ZHANG, J. G.; WEI, Z. J. Evolution of okara from waste to value added food ingredient: An account of its bio-valorization for improved nutritional and functional effects. **Trends in Food Science & Technology**, 116, pp. 669-680, 10.1016/j.tifs.2021.08.011. 2021.

GIRALDO, G. I.; CRUZ, C. D.; SANABRIA, N. R. Propiedades físicas del jugo de uchuva (*Physalis peruviana*) clarificado en función de la concentración y la temperatura, **Información Tecnológica**, 28, pp 133-142. 2017.

GONZALEZ-SOTO, M.; WOODS, S. E.; MACLEOD, B.; WRIGHT, D. C.; MUTCH, D. M. A moderate-fat diet containing soy protein does not differentially impact energy balance in male and female mice compared to dairy protein. **Nutrition Research**, 113, pp 59-65, ISSN 0271-5317, https://doi.org/10.1016/j.nutres.2023.03.005. 2023.

GUIMARÃES, R. M.; IDA, E. I.; FALCAO, H. G.; DE REZENDE, T. A. M.; SILVA, J. D. S.; ALVES, C. C. F.; DE SILVA, M. A. P.; EGEA, M. B. Evaluating technological quality of

okara flours obtained by different drying processes. **Lebensmittel- Wissenschaft und -Technologie- Food Science and Technology**, 123, 109062. 2020.

HACKLER, L. R. Methods of measuring protein quality: a review of bioassay procedure. **Journal Cereal Chemist**, v. 54, n. 5, p. 984-995. 1979.

HAN, Z. T.; LONG, W. M.; ZHANG, T. H.; DONG, Z. Y.; YAN, J. X. Application of xanthan gum and konjac gum to improve the texture, rheological properties and microstructure of Oviductus Ranae gel. **International Journal of Biological Macromolecules**, 222, Part B, pp 2709-2718. ISSN 0141-8130, https://doi.org/10.1016/j.ijbiomac.2022.10.052. 2022.

HARIKRISHNAN, V. S. Ethical issues in animal experimentation, Editor(s): MOHANAN, P. V. In: Woodhead Publishing Series in Biomaterials, **Biomedical Product and Materials Evaluation**, Woodhead Publishing, pp. 355-372, ISBN 9780128239667, https://doi.org/10.1016/B978-0-12-823966-7.00004-9. 2022.

HAWKINS, P.; GOLLEDGE, H. D. R. The 9 to 5 Rodent – Time for Change? Scientific ahesisnd animal welfare implications of circadian and light effects on laboratory mice and rats, **Journal of Neuroscience Methods**, 300, pp 20 25.https://doi.org/10.1016/j.jneumeth.2017.05.014. 2018.

HOYOS, J.; VILLADA, H.; FERNANDEZ, A.; ORTEGA-TORO, R. Parámetros de Calidad y Metodologías para Determinar las Propiedades Físicas de Alimentos Extruidos para Peces. **Información Tecnológica**, 28, p. 101-114. 2017.

IVANOVA, K.; DENKOVA, R.; KOSTOV, G.; PETROVA, T.; BAKALOV, I.; RUSCOVA, M.; PENOV, N. Extrusion of brewers' spent grains and application in the production of functional food. Characteristics of spent grains and optimization of extrusion. **Institute of Brewing & Distilling**, 123, 544–552. https://doi.org/10.1002/jib.448. 2017.

JEYAKUMAR, S. M.; LOPAMUDRA, P.; PADMINI, S.; BALAKRISHNA, N.; GIRIDHARAN, N.; VAJRESWARI, A. Fatty acid desaturation index correlates with body mass and adiposity indices of obesity in Wistar NIN obese mutant rat strains WNIN/Ob and WNIN/GR-Ob. **Nutrition & Metabolism**. v.6. n. 27. 10.1186/1743-7075-6-27. 2009.

KAMBLE, D. B.; RANI, S. Bioactive components, in vitro digestibility, microstructure and application of soybean residue (okara): A review. **Legume Science**, v. 2, n. 1, e32. 2020.

LEGENDRE, L. F. Oral disorders of exotic rodents. **Vet Clin North Am Exot Anim Pract**, v. 6, n. 3, pp/ 601-28. doi: 10.1016/s1094-9194(03)00041-0. PMID: 14534975; PMCID: PMC7128658. 2003.

LOPES, G. K. P.; ZANELLA, H. G.; SPESSATO, L.; RONIX, A.; VIERO, P.; FONSECA, J. M.; YOKOYAMA, J. T. C.; CAZETTA, A. L.; ALMEIDA, V. C. Steam-activated carbon from malt bagasse: optimization of preparation conditions and adsorption studies of sunset yellow food dye. **Arabian Journal of Chemistry**, 14 https://doi.org/10.1016/j. arabjc.2021.103001. 2021.

MALENICA, D.; KASS, M.; BHAT, R. Sustainable Management and Valorization of Agri-Food Industrial Wastes and By-Products as Animal Feed: For Ruminants, Non-Ruminants and as Poultry Feed. **Sustainability**, v. 15, n. 1, 117. https://doi.org/10.3390/su15010117. 2023.

MATEOS-APARICIO, I.; REDONDO-CUENCA, A.; VILLANUEVA-SUÁREZ, M. J. Isolation and characterization of cell wall polysaccharides from legume by-products: Okara (soymilk residue), pea pod and broad bean pod. **Food Chemistry**, v. 122, n. 1, pp. 339-345. ISSN 0308-8146, https://doi.org/10.1016/j.foodchem.2010.02.042. 2010.

MARTINS MULITERNO, M.; RODRIGUES, D.; SANCHES DE LIMA, F.; IOUKO IDA, E.; KUROZAWA, L. E. Conversion/degradation of isoflavones and color alterations during the drying of okara, **LWT**, v. 75, pp. 512-519, ISSN 0023-6438, https://doi.org/10.1016/j.lwt.2016.09.031. 2017.

MUSSATTO, S. I.; DRAGONE, G.; ROBERTO, I. C. Brewers' spent grain: Generation, characteristics and potential applications. **Journal of Cereal Science**, v. 43, n. 1, p. 1–14. https://doi.org/10.1016/j.jcs.2005.06.001. 2006.

NATIONAL RESEARCH COUNCIL COMMITTEE. **Annual Report**. Washington, DC: The National Academies Press. 1995.

OIKE, H.; KOHYAMA, K.; MOCHIZUKI-KAWAI, H; AZAMI, K. Food hardness influences the progression of age-related hearing loss in mice. **Experimental Gerontology**. 149, 111335, ISSN 0531-5565, https://doi.org/10.1016/j.exger.2021.111335. 2021.

OLIVEIRA SILVA, M.; CHAVES ALMEIDA, F. L.; NEVES DA PAIXÃO, R.; CÂNDIDO DE SOUZA, W. F.; DE LUNA FREIRE, K. R. PEREIRA DE OLIVEIRA, C. Preparation and characterization of churro dough with malt bagasse flour. **International Journal of Gastronomy and Food Science**, v. 27, 100427, ISSN 1878-450X, https://doi.org/10.1016/j.ijgfs.2021.100427. 2022.

OSTERMANN-PORCEL, M. V.; QUIROGA-PANELO, N.; RINALDONI, A. N.; CAMPDERROS, A. E. Incorporation of okara into gluten-free cookies with high quality and nutritional value. **Journal of Food Quality**, 4071585. 2017.

OSTERMANN-PORCEL, M. V.; RINALDONI, A. N.; RODRIGUEZ-FURLAN, L. T.; CAMPDERROS, M. E. Quality assessment of dried okara as a source of production of gluten-free flour. **Journal of the Science of Food and Agriculture**, v. 97, n. 9, pp. 2934–2941. 2016.

PAPAGEORGIOU, M.; SKENDI, A. Introduction to cereal processing and by-products. In: **Sustainable Recovery and Reutilization of Cereal Processing By-Products**; Elsevier: Amsterdam, pp. 1–25, 2018.

PRÉSTAMO, G.; RUPÉREZ, P.; ESPINOSA-MARTOS, I. et al. The effects of okara on rat growth, cecal fermentation, and serum lipids. **Eur Food Res Technol**. 225, 925–928. https://doi.org/10.1007/s00217-006-0497-4. 2007.

RIGO, M.; BEZERRA, J. R. M. V.; RODRIGUES, D. D.; TEIXEIRA, A. M. Physical-chemical and sensory characterization of cookies added with brewer's spent grain flour as fiber supply. Ambiência, 13. https://doi.org/10.5935/ambiencia.2017.01.03. 2017.

REDONDO-CUENCA, A.; VILLANUEVA-SUÁREZ, M. J.; MATEOS-APARICIO, I. Soybean seeds and its by-product okara as sources of dietary fibre: measurement by AOAC and English methods. **Food Chemistry**. 108, pp.1099–1105. 2008.

ROUDAUT, G. Water Activity and Physical Stability. In: BARBOSA-CÁNOVAS, G. V.; FONTANA JR., A. J.; SCHMIDT, S. J.; LABUZA, T. P.; Eds. **Water Activity in Foods: Fundamentals and Applications**, Second Edition. Chapter 10. John Wiley & Sons, Inc. 2020.

SILVA, W. S.; ARBAIZA, T. F.; CARCELÉN, F. C; LUCAS, O. A. Evaluación Biológica en ratas de Laboratorio (*Rattus norvegicus*) de Fuentes Proteicas usadas en alimentos comerciales para perros. **Revista de Investigaciones Veterinarias del Perú**, v. 14, n. 1, p. 18-23. http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1609-91172003000100004&lng=es&tlng=es. 2003.

STEPIEN, A.; GRZYB, K. Comparison of critical storage parameters of the powders containing soy protein isolate and inulin, based on the concepts: Water activity and Temperature of glass transition. **International Journal of Biological Macromolecules**, 230, https://doi.org/10.1016/j.ijbiomac.2023.123174. 2023.

SWALLAH, M. S.; FAN, H.; WANG, S.; YU, H.; PIAO, C. Prebiotic impacts of soybean residue (okara) on eubiosis/dysbiosis condition of the gut and the possible effects on liver and kidney functions. **Molecules**, v. 26, n. 2, p. 326. https://doi.org/10.3390/molecules26020326. 2021.

TAPIA, M.; ALZAMORA, S; CHIRIFE, J. Effects of Water Activity (a_w) on Microbial Stability: As a Hurdle in Food Preservation. In: BARBOSA-CÁNOVAS, G. V.; FONTANA JR., A. J.; SCHMIDT, S. J.; LABUZA, T. P. Eds. Water Activity in Foods: Fundamentals and Applications, 2 ed. Chapter 10. John Wiley & Sons, Inc. 2020.

WARWICK, Z. S.; SYNOWSKI, S. J. Effect of Food Deprivation and Maintenance Diet Composition on Fat Preference and Acceptance in Rats. **Physiology & Behavior**, v. 68, p. 235-239. 1999.

WATERS, D. M.; JACOB, F.; TITZE, J.; ARENDT. E. Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain enrichment. **European Food Research and Technology**, v. 235, p. 767–778. https://doi.org/10.1007/s00217-012-1805-9. 2012.

WEISKIRCHEN, S.; WEIPER, K.; TOLBA, R. H.; WEISKIRCHEN, R. All You Can Feed: Some Comments on Production of Mouse Diets Used in Biomedical Research with Special Emphasis on Non-Alcoholic Fatty Liver Disease Research. **Nutrients**, v. 12, n. 163. https://doi.org/10.3390/nu12010163. 2020.

Recebido: 27 jun. 2024.
Aprovado: 31 mar. 2025.
DOI: 10.3895/rebrapa.v15n1.18763
Como citar:
CAMPDERRÓS, M. E. et al. Valorization of industrial byproducts, okara, and beer bagasse to design balanced laboratory animal feed. **Brazilian Journal of Food Research,** Campo Mourão, v. 15 n. 1, p. 20-43, jan./jun. 2024. Disponível em: https://periodicos.utfpr.edu.br/rebrapa

Correspondência:
María Victoria Ostermann-Porcel
Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. Instituto de Investigaciones en Tecnología Química (INTEQUI). Ejército de los Andes 950. San Luis. Argentina.