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Evaluation of the physicochemical and technological characteristics of extruded feed for rodents with gluten

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ABSTRACT
Animals such as rodents are used as a tool for experimental studies in various areas of research. Therefore, in order to enable future in vivo tests of the impact of gluten on rodent health, the following paper aims to produce and technologically characterize rodent feed with the addition of gluten. A single-screw extruder was used to produce the samples. Four formulations were tested, increasing the amount of wheat added to each mix. The physicochemical and technological properties of the feed were determined, including a centesimal analysis, expansion index, water absorption index (WAI) and water solubility index (WSI). The results showed that the addition of wheat increased the crude protein content, a parameter that had a major influence on the expansion index and WSI. In the case of the expansion index, the increased protein content was possibly the cause of the increase in this parameter; for the WSI, the presence of proteins had the same effect, due to the denaturation of these biopolymers. In general, the results are positive in terms of nutritional composition, but more studies are needed to understand the consequences of adding gluten to feed.

Keywords: Physicochemical, Rodents, Gluten.

1 INTRODUCTION

Animals such as rodents are used as a tool for experimental studies in various areas of research, with the purpose of contributing to the advancement of science and technology. Proper nutrition for rodents is critical to their health and well-being, as well as directly influencing factors such as optimal growth, reproduction, and immune function. The composition of the diet plays an important role in meeting the nutritional needs of rats, impacting everything from animal husbandry to research laboratories, such as laboratory animals. In addition, the similarity between the gastrointestinal system of rodents and humans makes it feasible for *in vivo research* (REEVES et al., 1993; MOURA, 2014; TILOCCA, 2017).

Commercially, the feeds produced for rodents have a unique formulation, but the type of raw material and its composition may vary according to the market or operational adjustments. For the feeding of these laboratory animals, extruded feed is one of the most used in this niche (MOURA, 2014).

Extrusion is a method that has been employed since the 1930s, and applied commercially in the following decade. In the beginning, the result of this process was usually salty snacks extruded from corn meal (ROCKEY, PLATTNER & SOUZA, 2010).

The extrusion process is based on the union of several unit operations in the same equipment, which consists of modifying the physicochemical parameters of the raw material from the combination of a series of variables, such as mixing, cooking time, drying, molding, among others. This process is based on the transformation of a solid matter into a fluid from the application of heat and mechanical work, followed by molding through an outlet hole (LOPES-DA-SILVA, SANTOS & CHOUPINA, 2015; ROCKEY, PLATTNER & SOUZA, 2010),

The choice of ingredients exerts a significant influence on the texture, uniformity, and quality of the finished product. The use of starchy products promotes the gelatinization of starch, which increases viscosity, while protein-rich ingredients interfere with the elasticity of the product (ROCKEY, PLATTNER & SOUZA, 2010)

Gluten is a protein component from cereals such as wheat, barley and rye, it is composed of several proteins with gliadin and glutenin being the main constituents. These proteins are difficult to digest by enzymes in the intestine and pancreas, which for some individuals may be a pathology due to hypersensitivity or the cause of allergic reactions to gluten (BIESIEKIERSKI, 2017; NOBRE, SILVA & CABRAL, 2007).

Thus, this work aimed at the elaboration and technological characterization of a diet for laboratory rodents with the addition of gluten for later *in vivo studies*.

2 MATERIALS AND METHODS

For the formulation of the ration, the following raw materials were used: corn grits (Nutrimilho, Maringá, PR), toasted soybean meal (Cocamar, Maringá, PR), rice bran (Arrozmil, Maringá, PR), dark biscuit wheat flour (Cocamar, Marialva, PR), soybean oil (Cocamar, Maringá, PR), sodium chloride (Cisne, São Paulo, SP), dicalcium phosphate (Labsynth, Diadema, SP), calcium carbonate (Labsynth, Diadema, SP), vitamin and mineral premix for rodents PX1577 (Nucleopar, Mandaguari, PR). The proportions used are shown in Table 1.

Table 1 - Composition of the diets produced.

Ingredient	T0 (%)	T1 (%)	T2 (%)	T3 (%)
Corn Grits	61,4	61,4	33,4	0,00
Soybean meal	14,1	14,1	14,1	14,1
Wheat flour	0,00	14,0	42,0	70,0
Rice bran	14,0	0,00	0,00	0,00
Soybean oil	4,00	4,00	4,00	4,00
Sodium chloride	0,68	0,68	0,68	0,68
Dicalcium phosphate	1,14	1,14	1,14	1,14
Calcium carbonate	2,50	2,50	2,50	2,50
Vitamin-enriched premix	1,00	1,00	1,00	1,00
Premix mineral	1,18	1,18	1,18	1,18

The raw materials were weighed on an M214Ai analytical scale (Bel Equipamentos, Piracicaba, SP) or DST-30/C-DM digital scale (Triunfo, São Paulo, SP) and then homogenized. The mixture was added 20% (w/v) of water in relation to the final mass of product to be extruded and homogenized again. To ensure fluidity in the feed, the mixtures were screened for the disaggregation of lumps formed in the previous step.

According to Monteiro et al. (2016), an IMBRA RX50 single-screw extruder (INBRAMAQ, Ribeirão Preto, SP) with a diameter of 50 mm and a length of 200 mm was used. The die used had a 6 mm diameter outlet, in addition the motor amperage was kept at 20A, the feed rate 12 g/s and the screw speed 90 rpm.

The physicochemical analysis (dry matter, mineral matter, crude protein, ether extract and crude fiber) were performed by the Laboratory of Food Analysis and Animal Nutrition (LANA) of the State University of Maringá according to an approximate analysis of the food by means of the Weende System (centesimal composition) (Andriguetto, 1981). The moisture content was obtained according to equation 1 and the non-nitrogenous extract according to equation 2.

$$M\ (\%) = 100 - DM \quad (1)$$

$$NNE\ (\%) = DM - MM - CP - EE - CF \qquad (2)$$

Where:

M - moisture (%);

DM - dry matter (%);

NNE - non-nitrogenous extract (%);

MM - mineral matter (%);

CP - crude protein (%);

EE - ether extract (%);

CF - crude fiber (%).

The expansion index was determined by the ratio between the mean diameter of the samples and the diameter of the extruder die according to Mercier, Linko and Harper (1998), according to equation 3. Measurements were made with an analog caliper (Jomarca, Guarulhos, SP) with 20 kibbles of each formulation.

$$EI = \frac{D_E}{D_d} \qquad (3)$$

Where:

DE - extruded diameter (cm);

DD - diameter of the die (cm).

For the water absorption index (WAI) and water solubility index (WSI), 2.5 g of ground sample (passing through mesh 60) were suspended in 30 ml of water at room temperature in a falcon tube that was stirred at 10-minute intervals for a total period of 30 minutes, then the suspension was centrifuged for 15 minutes at 3400 rpm in a Q222T2 centrifuge (Quimis, Diadema, SP). The supernatant was poured into evaporation dishes to dry in a forced convection oven at 110ºC for 2 hours. The precipitate of the tube was weighed for the calculation of WAI and the dry supernatant for the calculation of WSI, according to equations 4 and 5, respectively (ANDERSON, CONWAY & PEPLINSKI, 1970). The analyses were performed in triplicate for each formulation.

$$WAI\ (g_{gel}/g_{sample}) = \frac{P}{S \times \frac{(100 - M)}{100}} \qquad (4)$$

Where:

P - mass of the precipitate (g);

S - sample mass (g);

M - Sample moisture (%).

$$WSI(\%) = \frac{DS}{S} \times 100 \quad (5)$$

Where:

DS - dry supernatant mass (g);

S - mass of the sample (g).

The statistical analysis of the results obtained was performed using analysis of variance (ANOVA), Tukey's test for comparison of means at the 5% level of significance, and correlation analysis using the Sisvar *5.6 software*.

3 RESULTS AND DISCUSSION

Table 2 - Results of the physicochemical characterization of the extruded products.

Parameter	T0	T1	T2	T3
Moisture (%)	3.97±0.35A	4.27±0.07A	4.52±0.37A	5,74±0,10b
Mineral Matter (%)	6.09±0.11c	4.41±0.25A	4.52±0.36A	5,41±0,10b
Crude protein (%)	14.16±0.14a	14,79±0,07b	16.63±0.26c	18,81±0,09d
Ether extract (%)	3.61±0.18A	3.67±0.62a	4.40±0.39A	4.48±0.43s
Crude fiber (%)	2.66±0.08A	2.63±0.13A	2.92±0.17A	2.70±0.22A
Non-Nitrogenous Extract (%)	69.51±0.08C	70.23±0.54c	67,01±0,58b	62.85±0.58A

Mean ± standard deviation. Results with different letters on the same line are significantly different by Tukey's test ($p \leq 0.05$).

Table 3 - Results of the technological characterization of extruded products.

Parameter	T0	T1	T2	T3
Expansion Index	1.99±0.07A	2,11±0,10b	2.45±0.16c	2,61±0,13d
WAI (g $_{gel}$/g$_{sample}$)	3,63±0,07b	3.19±0.08A	3.43±0.07ab	3,59±0,17b
WSI (%)	8.98±0.25A	9,77±0,19b	10,08±0,20bc	10.53±0.09c

Mean ± standard deviation. Results with different letters on the same line are significantly different by Tukey's test ($p \leq 0.05$).

Table 4 - Results of the correlation analysis between the parameters studied.

	[Wheat]	**M**	**MM**	**CP**	**EE**	**CF**	**NNE**	**EI**	**WAI**	**WSI**
[Wheat]	1,00	-	-	-	-	-	-	-	-	-
M	0,95	1,00	-	-	-	-	-	-	-	-
MM	-0,18	0,00	1,00	-	-	-	-	-	-	-
CP	1,00	0,97	-0,10	1,00	-	-	-	-	-	-
EE	0,94	0,80	-0,22	0,93	1,00	-	-	-	-	-
CF	0,39	0,09	-0,38	0,35	0,67	1,00	-	-	-	-
NNE	-0,95	-0,96	-0,13	-0,97	-0,88	-0,28	1,00	-	-	-
EI	0,99	0,89	-0,24	0,98	0,98	0,53	-0,92	1,00	-	-
WAI	0,22	0,28	0,86	0,29	0,27	0,08	-0,49	0,21	1,00	-
WSI	0,94	0,88	-0,47	0,92	0,86	0,36	-0,80	0,93	-0,12	1,00

Legend: [Wheat] = concentration of wheat used in the formulation, M = moisture, MM = mineral matter, CP = crude protein, EE = ether extract, CF = crude fiber, NNE = non-nitrogenous extract, EI = expansion index, WAI = water absorption index, WSI = water solubility index.

The moisture content of the diets did not show a significant difference between the samples T0, T1 and T2 with a significance level of 5%, and T3 was the only statistically different, so we can infer that the variation in the amount of wheat did not influence this parameter. For mineral matter, T0 presented the highest value, in addition to being statistically different from all other values, T1 and T2 did not have significant differences between them with the lowest values found and, finally, T3 was significantly different from the other three formulations, presenting an intermediate value.

The protein results showed that the concentration of wheat was an important factor in its variation, presenting a very strong correlation ($r = 1.00$), that is, the concentration of crude protein is directly proportional to the concentration of wheat flour. In addition, there was a significant difference with a 95% confidence level between all formulations, reinforcing that the correlation mentioned above is real. The results presented were expected since wheat has between 8% and 15% of proteins, 85 to

90% of which is gluten still in the form of gliadin and glutenin (BIESIEKIERSKI, 2017). In addition, the other components of the formulation may have contributed to this protein content, soybean, for example, contains 30% to 45% of proteins in its composition (BORDINGNON & MANDARINO, 1994), in bran this content increases, with the manufacturer guaranteeing a minimum content of 46% of crude protein.

The ether extract results did not show significant difference between any of the compositions, a fact explained by the addition of the same fraction of soybean oil in all formulations. The crude fiber content also did not show significant differences between any of the formulations ($p > 0.05$), probably because a starchy matter was replaced by a similar one, in this case corn grits replaced by wheat flour.

The non-nitrogenous extracts or non-structural carbohydrates had a decrease in the content with the increase in wheat concentration ($r = -0.95$), this relationship may be explained by the relationship between non-nitrogen extracts and crude protein content, which shows the same trend ($r = -0.97$). In addition, T0 and T1 did not differ significantly from each other for non-nitrogen extracts, whereas T2 and T3 were significantly different among all formulations.

There was a significant difference between all formulations for the expansion index of extruded products ($p \leq 0.05$). In addition, it is possible to observe the behavior of increase in the expansion index with the increase in the proportion of wheat flour in the mixture ($r = 0.99$), i.e., the more wheat added, the greater the expansion of the extruded. According to Arhaliass et al. (2009), the protein content positively influences the expansion of the product, in order to alter the distribution of water in the matrix structure, which contributes to the extensional properties of the extrudate, a fact that is reinforced by the very strong correlation ($r = 0.98$) between the expansion index and the protein concentration.

The expansion index showed an inversely proportional behavior to the non-nitrogenous extract ($r = -0.92$), which was basically composed of carbohydrates. This behavior is contradictory to what is found in the literature, Júnior et al. (2011) explain that the starch content directly influences the expansion index of the extruded, because when passing through the equipment, the carbohydrate gelatinization occurs, which helps in the expansion of the product. However, this fact is not observed in this study, and further analysis and studies are needed to understand the behavior found.

The water absorption index did not show a specific behavior in relation to wheat concentration. For Tukey's test, there was no significant difference between the results of T0, T2 and T3, and T1 and T2 also showed no difference between them. On the other hand, the results of the water solubility index showed a very strong correlation with the amount of wheat added to the formulation ($r = 0.94$), so the increase of this component increases the WSI of the feed, a behavior that suggests greater dextrinization of the product with a greater amount of wheat (KOWALSKI, MORRIS & GANJYAL, 2015, apud GOMEZ & AGUILERA, 1984).

A strong relationship between protein content and WSI was observed (r = 0.92), which suggests that the extrusion, in addition to gelatinizing the starch, also denatured the proteins present, i.e., weakened and broke the bonds of the biopolymers, which facilitates the penetration of water into the structures, increasing the WSI due to the greater exposure of hydrophilic groups (LUSTOSA, LEONEL & MISCHAN, 2009). Regarding the mean test, T2 did not show significant difference when compared with T1 and T3.

4 CONCLUSION

The addition of gluten in rodent feed had a significant impact on the physicochemical and technological properties of the final product, with emphasis on protein content, expansion index and water solubility index. The increase in wheat concentration was nutritionally positive, as it increased the amount of protein. However, this increase may have intensified the expansion of extruded products, which can be detrimental to the density of the product, decreasing it, making it difficult to package and transport the feed.

Another parameter influenced by wheat concentration and, consequently, protein content, was the water solubility index, which may have important implications for the digestibility and nutrient absorption of the diet. In conclusion, this study provides valuable insights into the impact of adding a gluten source to rodent feed, paving the way for future investigations into the impact of gluten on rodent health and well-being.

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