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Dehydrated Brewery Residue (DBR) can reduce the cost of rabbit production in Brazil, but affects performance, blood nutrients, and carcass characteristics

O Resíduo Desidratado de Cervejaria (RDC) pode reduzir o custo da produção de coelhos no Brasil, mas afeta o desempenho, os nutrientes sanguíneos e as características da carcaça

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Highlights

DBR reduces the cost of rabbit production.
Performance of growing rabbits is affected by DBR.
Rabbit meat tenderness is reduced by DBR.

Abstract

The study aimed to determine the chemical composition, apparent digestibility of dry matter, energy, and nutrients of Dehydrated Brewery Residue (DBR) for New Zealand White rabbits, as well as evaluate performance, biochemical and immunological blood parameters, carcass characteristics, and meat quality. Two experiments were carried out: digestibility and performance, both in a completely randomized design. In the digestibility experiment, 20 animals of mixed sexes were used, from 45 to 60 days of age, which consumed reference feed (RF) or test feed (TF - 70% RF + 30% DBR). In the performance experiment, 50 mixed-sex animals were used from 31 to 70 days of age. For digestibility, bromatological

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analyses were made of total dry matter (DM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), mineral matter (MM), organic matter (OM), calcium (Ca), phosphorus (P), gross energy (GE), hemicellulose, cellulose and lignin from samples of feces, feed (RF and TF) and experimental feed (DBR). In terms of performance, the treatments were the basal feed (BF) + four TF, made up of increasing levels of DBR inclusion (10, 20, 30, and 40%). Overall, the apparent digestibility coefficient (ADC) of DM was 64.68 ±7.91%, while the ADC of GE and CP were 67.39 ±7.79% and 80.64 ±8.99%, respectively, resulting in digestible energy and digestible protein contents of 3,081 ±356 kcal/kg and 17.97 ±2.00%. There was a linear reduction (P<0.05) in the final weight, daily weight gain, and daily feed intake, but there was a linear reduction in production costs (P<0.05) for both the 31- to 50-day phase and the 31- to 70-day phase. Circulating levels of calcium (P=0.013) and phosphorus (P=0.019) responded in a linear decreasing pattern to the experimental feeds. The same response was observed for slaughter weight, hot and cold carcass weight, yield of skin, head, commercial cuts, and relative weight of edible organs, as well as meat quality, in which the loss of water on thawing (P=0.004) and shear force (P=0.005) indicated less juiciness and less tenderness in the meat. Levels of 10 to 40% DBR in feed for rabbits from 31 to 70 days of age mitigate production costs, but result in a worsening of performance and reduce circulating levels of calcium and phosphorus, reduce carcass weight, commercial cuts, and edible organs, as well as increasing water loss on thawing, and reducing meat tenderness.

Key words: Alternative foods. Digestibility coefficient. Co-product. Rabbit farming. Brewery waste.

Resumo

O estudo teve como objetivo determinar a composição química, digestibilidade aparente da matéria seca, energia e nutrientes do Resíduo Desidratado de Cervejaria (RDC) para coelhos da raça Nova Zelândia Branco, bem como avaliar o desempenho, parâmetros sanguíneos, bioquímicos e imunológicos, características de carcaça e qualidade da carne. Foram realizados dois experimentos: digestibilidade e desempenho, ambos em delineamento inteiramente casualizado. No experimento de digestibilidade foram utilizados 20 animais de sexos mistos, de 45 a 60 dias de idade, que consumiram ração referência (RR) ou ração teste (RT: 70% RR + 30% RDC). No experimento de desempenho foram utilizados 50 animais, com idade entre 31 e 70 dias. Para digestibilidade foram feitas análises bromatológicas de matéria seca total (MS), proteína bruta (PB), fibra bruta (FB), fibra em detergente neutro (FDN), fibra em detergente ácido (FDA), extrato etéreo (EE), matéria mineral (MM), matéria orgânica (MO), cálcio (Ca), fósforo (P), energia bruta (EB), hemicelulose, celulose e lignina provenientes de amostras de fezes, ração (RR e RT) e alimento teste (RDC). Em termos de desempenho, os tratamentos foram alimentação basal (RR) + quatro RT, composto por níveis crescentes de inclusão de RDC (10, 20, 30 e 40%). De modo geral, o coeficiente de digestibilidade aparente (CDA) da MS foi de 64,68 ±7,91%, enquanto o CDA da EB e da PB foi de 67,39 ±7,79% e 80,64 ±8,99%, respectivamente, resultando em teores de energia digestível e proteína digestível de 3.081 ±356 kcal/kg e 17,97 ±2,00%. Houve redução linear (P<0,05) no peso final, ganho de peso diário e consumo diário de ração, mas houve redução linear nos custos de produção (P<0,05) tanto para a fase de 31 a 50 dias quanto para a fase de 31 a 70 dias. Os níveis circulantes de cálcio (P=0,013) e fósforo (P=0,019) responderam em padrão linear decrescente às rações experimentais. A mesma resposta foi observada para peso de abate, peso de carcaça quente e fria, rendimento de pele, cabeça, cortes comerciais e peso relativo de órgãos comestíveis, bem como qualidade da carne, em que a perda de água no descongelamento (P=0,004) e força de cisalhamento (P=0,005) indicou menor suculência e menor maciez na carne. Níveis de 10 a 40% DBR na ração para coelhos de 31 a 70 dias de idade atenuam os custos de produção, mas resultam em piora do desempenho e reduzem os níveis circulantes de cálcio e fósforo, reduzem o peso da carcaça, dos cortes comerciais e dos órgãos comestíveis, bem como aumentam a perda de água no descongelamento e reduzem a maciez da carne.

Palavras-chave: Alimento alternativo. Coeficiente de digestibilidade. Coproduto. Cunicultura. Resíduo de cervejaria.

Introduction

Every year, Brazilian agribusiness stands out among the sectors of the economy and gains more space in the country's Gross Domestic Product - GDP. Agroindustry is a segment of agribusiness represented by a conglomerate of activities that are related to each other. It involves the transformation of raw materials from agricultural production, such as agricultural inputs, and equipment used, and the conversion of raw materials into manufactured products, such as beverages and food (Centro de Estudos do Agronegócio [FGV], 2019). However, the agro-industrial sector faces great difficulties in correctly disposing of all the waste generated during production, which often ends up being disposed of inappropriately in the environment due to its large quantity.

Regarding some agro-industrial residues, a sustainable alternative is their use in animal feed, encouraging a possible integration between agroindustry and livestock farming, and creating sustainable production models that benefit both sectors. Beer is one of the many products that generate waste, and its production generates a co-product that can be used in animal feed, such as dried brewers' yeast and wet brewers' grain (WBG), also known as wet brewery byproduct or wet brewery waste. Drying WGB results in dehydrated brewery residue (DBR), which is easier to store and remains in good condition for longer, contributing to its use in feed production (Geron et al., 2007). The use of this waste minimizes animal feed costs, as it can be obtained throughout the year at a low cost (Santos et al., 2012).

Brewery waste is a co-product that has an abundant supply, low seasonality, as well as appreciable nutritional values, with average values of 90.85% organic matter (OM), 22.72% crude protein (CP), 4,724 kcal/kg gross energy (GE), 63.92% neutral detergent fiber (NDF), 23.97% acid detergent fiber (ADF) and 4.75% ether extract (EE) (Araujo et al., 2016).

Rabbits are animals with high dietary fiber requirements, but most production systems in Brazil are based on semi-intensive cage rearing, which requires the supply of complete feeds to meet the animals' requirements and prevent digestive disorders (Gidenne & Bellier, 2000). Due to the presence of a functional cecum, rabbits are better able to take advantage of coarse foods, as their active microbiota ferments a fraction of the dietary fiber, generating volatile fatty acids, vitamins, amino acids, and other essential nutrients for them (Araujo et al., 2016).

Although brewery waste is used in several countries to feed rabbits, there is still little information on the digestible nutrients and performance of rabbits fed beer waste made in Brazil. These values may be different from the digestible values obtained in other countries, where barley or rice is mainly used in the malting process, while in Brazil, Pilsen beer uses corn (Rogers et al., 1986). Also, its nutritional and digestible values depend on the type of beer brewed, and whether other cereals are used in its constitution (Velasco et al., 2009).

Given the above, this research aimed to determine the chemical and energy composition, apparent digestibility of dry matter, energy and nutrients, zootechnical performance, economic viability of production, blood parameters, carcass characteristics and meat quality of New Zealand White rabbits fed increasing levels of dehydrated brewery residue.

Materials and Methods

The experiment was carried out in the Cuniculture Sector, located at the Iguatemi Experimental Farm - FEI, belonging to the Agricultural Sciences Center of the State University of Maringá - UEM, in the state of Paraná, Brazil (23º21'S, 52º04'W and altitude of 564 m). All the experimental procedures were previously submitted to and approved by UEM’s Ethics Committee on Animal Use (CEUA/UEM) (Protocol no. 5729210120).

The brewery waste was obtained from a brewery called Cervejaria Araucária, located in the municipality of Maringá. It was bought when it was still wet, right after the brewing process of Pilsen beer, which is made up of Pilsen malt, hops, corn, water, and yeast. The material was transported in a plastic container with a lid to a place suitable for dehydration, which took place in the sun, on a previously cleaned and insulated concrete floor, remaining there for three days, and being turned five times a day. After drying, it was ground in a knife and hammer mill with a sieve fitted with 2.5 mm diameter holes, which resulted in a Geometric Mean Diameter (GMD) of 1022 µm.

Digestibility

Twenty New Zealand White rabbits were used, 10 males and 10 females, aged 45 days. The animals were housed individually in metabolism cages, all duly cleaned and disinfected, equipped with an automatic drinking fountain, a semi-automatic feeder, and a device for collecting feces. The animals were provided with feed and water at will, and feed consumption was calculated as the difference between the amount provided and the leftovers.

The reference feed (RF) was formulated from alfalfa hay, corn, soybean meal, wheat bran, amino acids, minerals, and vitamins, according to the requirements for growing rabbits (De Blas & Mateos, 2010), as shown in Table 1.

Table 1
Percentage and chemical composition of the reference feed (natural matter)

Ingredients (%)	RF%
Alfalfa hay	31.00
Wheat bran	29.63
Grain corn	25.00
DBR	0.00
Soybean meal	9.96
Soybean oil	1.648
Calcitic limestone	0.448
DL- methionine	0.233
L- lysine HCl	0.305
Vit. + Min. supplement1	0.500
Salt	0.976
Acidifier 2	0.300
TOTAL	100.00
Calculated chemical composition	
Dry matter (%)	88.63
Crude protein (%)	16.00
DE (Kcal/kg)	2400
ADF (%)	16.00
NDF(%)	29.16
EE (%)	5.34
Calcium (%)	0.800
Total phosphorus (%)	0.570
Met + Cis (%)	0.590
Lysine (%)	0.780

[1] Vitamin-mineral supplement: Composition per kg of product: vitamin A (min) – 600.000 IU; vitamin D (min) – 100.000 IU; vit. E – 8.000 mg; vit. K3 - 200 mg; vit. B1 - 400 mg; vit. B2 - 600 mg; vit. B6 - 200 mg; vit. B12 – 2.000 mcg; pantothenic acid – 2.000 mg; choline – 70.000 mg; Fe – 8.000 mg; Cu – 1.200 mg; Co - 200 mg; Mn – 8.600 mg; Zn – 12.000 mg; I - 64 mg; Se - 16 mg; Methionine – 120.000 mg; antioxidant -20.000 mg;

[2] Active ingredients based on Organic Acids: propionic acid, acetic acid, benzoic acid, caprylic acid, lauric acid, sorbic acid, capric acid, citric acid, formic acid, ammonium formate, and excipients.

After mixing the ingredients, the feed was dry pelletized using the Indústria Comercial Chavantes pelletizer, model 40 HP, with a capacity of 800 to 1,700 kg.h^{-1}, with a 4.5 mm grid and without the addition of steam, at an average temperature of 70ºC (60 to 80ºC), for around 50 seconds, and then bagged and stored in an adequate place.

The experimental design was entirely randomized, with ten replicates per treatment and one animal per experimental unit. The animals were assigned to experimental cages, the treatments of which were RF + test feed (TF), comprised of 70% RF and 30% experimental feed (DBR).

The experimental period lasted 15 days, with ten days for the animals to adapt to the metabolism cages and the feed, and five days for collecting feces. The methodology used was a total collection of feces, all collected and weighed daily at the same time, packed in plastic bags, and stored in a freezer (-18 °C).

After thawing the feces at room temperature, the samples from each animal were sent to the Animal Nutrition Laboratory of UEM's Animal Science Department and placed in a forced ventilation oven at 55ºC for 72 hours. After being homogenized, part of the sample (± 50%) was ground in a mill with a 1 mm sieve.

Samples of feces, feed (RF and TF) and DBR were submitted for analysis of total dry matter (DM), crude protein (CP), gross energy (GE), crude fiber (CB), neutral detergent fiber (NDF) and acid detergent fiber (ADF), lignin, cellulose, hemicellulose, ether extract (EE), mineral matter (MM) and organic matter (OM), according to Silva and Queiroz (2002), as well as calcium (Ca) and phosphorus (P) analyses.

The apparent digestibility coefficients (ADC) of DM, CP, GE, CF, NDF, ADF, lignin, cellulose, hemicellulose, EE, MM, OM, Ca, and P of the DBR were calculated using the methodology from Matterson et al. (1965). To obtain the digestible nutrient values, the respective ADC values were applied to the chemical composition of the feed being assessed.

Performance and blood tests

Fifty New Zealand White rabbits were used, 25 males and 25 females, from 31 to 70 days of age with an average initial weight of 665 grams. The animals were housed individually in metabolism cages equipped with an automatic drinking fountain and a semi-automatic feeder.

The experimental design was entirely randomized, with ten replicates per treatment (5 males and 5 females) and one animal per experimental unit. The animals were placed in cages in which the treatments consisted of basal feed (BF) + four test feeds (TF), with increasing levels of DBR inclusion (10, 20, 30, 40%). All the feeds were isoproteic and isoenergetic.

The BF was formulated from alfalfa hay, corn, soybean meal, wheat bran, amino acids, minerals, and vitamins, according to the requirements for growing rabbits (De Blas & Mateos, 2010), as shown in Table 2.

After mixing the ingredients, the experimental feeds were dry pelletized using the pelletizer from Indústria Comercial Chavantes, model 40 HP, with a capacity of 800 to 1,700 kg.h^{-1}, with a 4.5 mm grid and without the addition of steam, at an average temperature of 70 ºC (60 to 80 ºC), for around 50 seconds. Water and feed were provided at will.

The feed provided, the leftovers, and the animals were weighed at the start of the experiment (31 days), at 50 days, and at the end of the experiment (70 days). Daily feed intake (DFI) was calculated from the difference between the amount of feed supplied and the leftovers collected, divided by the days of supply. Daily weight gain (DWG) was calculated from the difference between the final weight (FW) and the initial weight (IW), divided by the evaluation days. The feed conversion ratio was calculated using the ratio between DFI and DWG.

Table 2

Centesimal composition of experimental feeds containing increasing dehydrated brewery residue (DBR) levels for growing rabbits

Ingredients (%)	DBR levels (%)				
	0	10	20	30	40
Alfalfa hay	31.00	31.00	31.00	31.00	31.00
Wheat bran	29.63	22.10	14.40	6.00	0.47
Grain corn	25.00	25.00	25.00	25.00	25.00
DBR	0.00	10.00	20.00	30.00	40.00
Soybean meal	9.96	7.91	6.03	4.80	0.76
Soybean oil	1.648	1.248	0.848	0.502	0.112
Calcitic limestone	0.448	0.448	0.448	0.448	0.448
DL- methionine	0.233	0.233	0.233	0.233	0.233
L- lysine HCl	0.305	0.285	0.261	0.243	0.196
Vit. + Min.[1] supplement	0.500	0.500	0.500	0.500	0.500
Salt	0.976	0.976	0.976	0.976	0.976
Acidifier[2]	0.300	0.300	0.300	0.300	0.300
TOTAL	100.00	100.00	100.00	100.00	100.00
	Calculated chemical composition				
Dry matter (%)	88.63	88.59	88.56	88.53	88.50
Crude protein (%)	16.00	16.00	16.00	16.00	16.00
DE (Kcal/kg)	2400	2400	2400	2400	2400
ADF (%)	16.00	16.00	16.00	16.00	16.00
NDF(%)	29.16	29.61	30.22	30.58	30.98
EE (%)	5.34	5.32	5.21	5.18	5.12
Calcium (%)	0.800	0.800	0.800	0.800	0.800
Total phosphorus (%)	0.570	0.570	0.624	0.682	0.739
Met + Cis (%)	0.590	0.590	0.590	0.590	0.590
Lysine (%)	0.780	0.780	0.780	0.780	0.780

[1] Vitamin-mineral supplement: Composition per kg of product: vitamin A (min) – 600.000 IU; vitamin D (min) – 100.000 IU; vit. E – 8.000 mg; vit. K3 - 200 mg; vit. B1 - 400 mg; vit. B2 - 600 mg; vit. B6 - 200 mg; vit. B12 – 2.000 mcg; pantothenic acid – 2.000 mg; choline – 70.000 mg; Fe – 8.000 mg; Cu – 1.200 mg; Co - 200 mg; Mn – 8.600 mg; Zn – 12.000 mg; I - 64 mg; Se - 16 mg; Methionine – 120.000 mg; antioxidant -20.000 mg;

[2] Active ingredients based on Organic Acids: propionic acid, acetic acid, benzoic acid, caprylic acid, lauric acid, sorbic acid, capric acid, citric acid, formic acid, ammonium formate, and excipients.

To check the economic viability of the feeds, the cost of the feed per kilogram of live weight gained (Yi) was determined, according to Bellaver et al. (1985).

$Y_i = (Q_i \times P_i) / G_i$

In which:

Yi= feed cost per kilogram of live weight gain in the i-th treatment;

Qi= amount of feed consumed in the i-th treatment;

Pi= price per kilogram of the feed used in the i-th treatment;

Gi= weight gain of the i-th treatment.

For the costs of the experimental feeds, the prices of inputs in the Maringá-PR region during the months of the experiment (July and August 2023) were used. As the DBR was donated, the price of the ingredient was not taken into consideration in the feed cost calculations.

When the animals reached 70 days of age, they were fasted for 4 hours and blood was collected at the time of slaughter. The blood samples were taken through the jugular vein and transferred to tubes containing EDTA or fluoride. After that, the plasma was separated by centrifuging them for 15 minutes at 3,000 rpm. Then, 3 mL of plasma was transferred to duly identified Eppendorf tubes and stored in a freezer (-18 °C) for later analysis. The entire slaughter process complies with CFMV Resolution no. 1000/2012 and under ORDER No. 47 OF MARCH 19, 2013 (MAPA/SDA), which establishes the Sensitization Methods for Humane Slaughter.

Analyses of glucose, total proteins, urea, triglycerides, total cholesterol, HDL, and LDL were carried out by the colorimetric method, using commercial kits, following the standard operating procedures (SOP) described therein.

Carcass characteristics and meat quality

After the slaughtering procedure, the skin was removed and evisceration was carried out. The viscera (heart, liver, and kidneys) and carcasses were weighed separately to determine the carcass yield (CY). In addition, the carcasses were divided into commercial cuts to obtain cut yields. For carcass weight and its relation with live weight, and the commercial cuts' weight and their relation with carcass weight, the hot carcass, with head and without edible viscera, was considered. The carcass characteristics analyzed were carcass weight and yield (CW and CY) and commercial cut weights and yields, respectively represented by forelimbs, hind limbs, loin, thoracic-cervical region, and head.

The pH of the loin muscle (*longissimus lumborum*) was measured with the hot carcass, 15 min after slaughter (pH15) and in the cooled carcass, kept in the cold room (1-2 °C) for 24 hours (pH24), using a HI 99163 digital portable pH meter (Hanna Instruments). For qualitative evaluation of the carcass, 24 hours after slaughter, 1.5 cm thick samples were taken from the loin muscle (*longissimus lumborum*) for subsequent measurement of water loss due to cooling, thawing, and cooking.

The color of the *longissimus lumborum* muscle was assessed 24 hours after slaughter using Minolta luminosity measurements (L*, a*, and b*), and a Konica Minolta's CR-400 portable colorimeter (settings: D65 illuminant; 0º viewing angle and 4 auto-average). The components L* (brightness), a* (red-green component), and b* (yellow-blue component) were expressed in the CIELAB color system. The cooked samples of *longissimus lumborum* were used to measure the shear force (kgf/cm^2). Six cylindrical sub-samples (diameter 1.27 cm) were taken longitudinally from each sample in the direction of the muscle fibers, according to the recommendations of Ramos and Gomide (2007). The analyses were carried out on a Stable Micro System TA-XT2i texturometer, attached to the Warner-Bratzler Shear Force probe and the Texture Expert Exponent - Stable Micro Systems software.

Statistical analysis

The UNIVARIANTE procedure was applied to assess the presence of outliers among the variables. The normality of the experimental errors and the homogeneity of variances between treatments for the various variables were previously assessed using the Shapiro-Wilk and Levene tests (Statistical Analysis System – SAS Inst. Inc., Cary, NC, USA), respectively.

The analysis of variance (ANOVA) was carried out using the General Linear Models (GLM) procedure of the Statistical Analysis System software (SAS Inst. Inc., Cary, NC, USA). For the performance variables (final weight, daily weight gain, daily feed intake, feed conversion ratio, and production cost), initial weight was used as a covariate, while for the carcass variables, slaughter weight was used as a covariate.

The degrees of freedom for the levels of DBR in the feed were divided into orthogonal polynomials to obtain the most appropriate regression equations. A significance level (P) of 0.05 was adopted for all analyses.

Results and Discussion

The total contents, apparent digestibility coefficients (ADC), and digestible contents of dry matter, nutrients, and energy of the DBR are shown in Table 3. The total contents of DM, CP, and gross energy were 94.59%, 22.28%, and 4,472 kcal/Kg respectively, while the total digestible contents for the same nutrients were 61.18%, 17.97%, and 3,081 kcal/Kg.

Table 3

Bromatological composition, apparent digestibility coefficients (ADC), and digestible contents of dry matter, energy, and nutrients of dehydrated brewery residue (DBR) for growing rabbits (in natural matter)

Variables	Total content	ADC ±SD[1] (%)	Digestible contents ±SD[1]
Dry matter (%)	94.59	64.68 ±7.91	61.18 ±7.48
Organic matter (%)	91.41	67.81 ±8.18	61.98 ±7.48
Mineral matter (%)	3.18	69.11 ±6.30	2.20 ±0.20
Calcium (%)	0.33	39.88 ±2.23	0.13 ±0.04
Phosphorus (%)	0.55	41.15 ±4.03	0.22 ±0.03
Gross energy (kcal/kg)	4,572	67.39 ±7.79	3,081 ±356
Crude protein (%)	22.28	80.64 ±8.99	17.97 ±2.00
Ether extract (%)	4.78	85.64 ±4.38	4.09 ±0.30
Crude fiber (%)	10.01	81.92 ±11.86	8.20 ±1.19
Neutral detergent fiber (%)	53.13	49.55 ±4.71	26.33 ±2.50
Acid detergent fiber (%)	20.32	24.67 ±7.44	5.01 ±1.51
Hemicellulose (%)	32.81	67.43 ±10.14	22.13 ±3.33
Lignin (%)	15.52	35.24 ± 7.09	5.47 ± 1.10
Cellulose (%)	4.79	57.15 ± 8.14	2.74 0.39

[1] - Standard deviation.

The bromatological composition of DBR determined in this study was similar to that obtained by Araujo et al. (2016), whose reported DM, OM, GE, CP, EE, NDF, and ADF values were 89.55%; 90.85%; 4723.83kcal/kg; 22.72%; 1.77%; 4.75%; 63.92% and 23.67%, respectively, based on natural matter. However, when compared with the results found by Lima et al. (2017), only the DM, OM, GE, NDF, and ADF values were similar, corresponding to 90.43%; 5.30%; 4803.50kcal/kg; 51.72%, and 22.08% respectively, while the CP (37.9%) and EE (8.1%) values reported by Lima et al. (2017) were higher than those obtained in this study, 22.28% and 4.78% respectively.

This high variability in the data compared to other authors is because there are variations in the types and quantities of grains used in the beer production, as well as the processing adopted by the industry for this production (Araujo et al., 2016). In the case of the DBR evaluated in this study, it is waste from the production of craft pilsen beer, whose basic ingredients are pilsen malt, hops, corn, water, and yeast.

The crude protein content of DBR is higher than that of alfalfa hay (17%), but lower than that of soybean meal (45%). However, when feed costs are considered, DBR becomes a viable and high-quality option to replace both feeds (Rostagno et al., 2017).

The apparent digestibility coefficient of gross energy (ADCGE) of DBR was 67.39 ± 7.79, resulting in 3,081 kcal/kg of digestible energy, similar to the values obtained by Lima et al. (2017) of 70.2% and 3,371kcal/kg, respectively. All the other digestibility coefficients had discrepant results when compared to those reported by Araujo et al. (2016) and Lima et al. (2017), even though the authors used animals in the same age range. In the present study, the crude protein digestibility coefficient (ADCCP) was 80.64 ± 8.99%, while Araujo et al. (2016) reported an ADCCP of 71.06%, and Lima et al. (2017) obtained an ADCCP of 41%.

When the ADC values for DM, OM, CP, and GE are compared with those published by Fernandez-Carmona et al. (1996), the values are closer. For these authors, the corresponding values were 58%, 59.4%, 79.3%, and 62.2%, respectively. Furthermore, if we analyze the values of the digestibility coefficients of the NDF and ADF (49.55 ±4.71 and 24.67 ±7.44) of the residue with the values reported by Maertens and Salifou (1997), of 39.6% and 17.5%, respectively, these are the results that are closest to the data obtained.

The hemicellulose, cellulose, and lignin contents of DBR (32.81%, 4.79%, and 15.52%, respectively) indicate that this material can be used as the main source of insoluble fiber in rabbit diets since it has moderate lignin and cellulose contents that do not predispose the animals to intestinal disorders such as diarrhea and others (Condé et al., 2014). However, when analyzing the performance results of the rabbits with the residue, a gradual deterioration was observed as the inclusion of DBR in the diet increased.

In terms of performance, rabbits fed diets containing increasing levels of DBR from 31 to 50 days of age showed a linear reduction ($P<0.05$) in final weight, daily weight gain, and daily feed intake, as shown in Table 4. Even so, there was a linear reduction in the cost of production ($P<0.05$) for both the 31 to 50-day phase and the 31 to 70-day phase.

For the period from 31 to 70 days of age, the same behavior was observed for the above-mentioned variables, showing that the inclusion of levels of DBR above 10% in rabbit diets leads to a deterioration in the productive performance of the animals after weaning, even if the diets are isonutritive.

The deterioration in performance may have been due to the sharp decrease in feed consumption, which consequently affected the weight gain and final weight of the rabbits. Although there was no effect of the treatments on the feed conversion in any of the phases evaluated, both daily feed consumption and daily weight gain decreased linearly throughout the treatments, which does not affect the value obtained for conversion, calculated using the ratio between these two variables.

The same was found by Klinger et al. (2021), in which the weight of rabbits, in both periods (35 to 56 days and 56 to 77 days), gradually decreased with the inclusion of brewery residue, so that daily weight gain and daily feed intake also decreased in the same proportion, but there was no effect on feed conversion for each period. However, when analyzing feed conversion for the period as a whole, there was a significant response ($P<0.05$).

Table 4
Performance variables of rabbits fed diets containing increasing levels of dehydrated brewery residue (DBR)

Variables	DBR levels (%)					SEM[1]	P-value
	0	10	20	30	40		
			31 to 50 days				
Initial weight (g)	666	678	673	681	644	-	-
Final weight (g)[2]	1398	1274	1283	1344	1158	17.54	0.018
Daily weight gain (g)[3]	37	30	31	33	26	0.86	0.014
Daily feed intake (g)[4]	80	68	67	72	56	1.35	<0.001
Feed conversion ratio	2.20	2.33	2.24	2.20	2.21	0.03	0.829
Production cost (R$/kg GP)[5]	2.70	2.65	2.34	2.12	1.88	0.03	<0.001
			31 to 70 days				
Initial weight (g)	666	678	673	681	644	-	-
Final weight (g)[6]	2300	2008	2157	2164	1892	25.90	0.003
Daily weight gain (g)[7]	82	67	76	74	62	1.29	0.003
Daily feed intake (g)[8]	188	179	185	180	154	3.09	0.039
Feed conversion ratio	2.29	2.67	2.44	2.43	2.47	0.02	0.111
Production cost (R$/kg GP)[9]	2.81	2.78	2.55	2.49	2.10	0.02	<0.001

[1] Standard error of the mean.
[2] Linear effect (P=0.081): $Y= 1.373.40 - 4.10x$ ($r^2=0.52$).
[3] Linear effect (P=0.033): $Y= 35.20 - 0.19x$ ($r^2=0.55$).
[4] Linear effect (P=0.005); $Y= 77.40 - 0.44x$ ($r^2= 0.64$).
[5] Linear effect (P<0.001); $Y= 2.77 - 0.02x$ ($r^2= 0.97$).
[6] Linear effect (P=0.020); $Y= 2.236.20 - 6.60x$ ($r^2= 0.44$).
[7] Linear effect (P=0.010); $Y= 78.80 - 0.33x$ ($r^2= 0.45$).
[8] Linear effect (P=0.084); $Y= 190.60 - 0.67x$ ($r^2= 0.62$).
[9] Linear effect (P<0.001); $Y= 2.88 - 0.02x$ ($r^2= 0.90$).

Contrary to the results found in this study, Araujo et al. (2016) observed no deterioration in the performance of New Zealand White rabbits when fed DBR, with no loss in weight gain or daily feed intake. The authors pointed out that DBR can be included in the diet up to 25% without affecting the performance of the animals from 32 to 50 days of age and also from 32 to 70 days of age. However, a quadratic behavior was observed in the feed conversion ratio variable, with the worst result estimated at 16.95% inclusion, which did not occur with the same variable in this study, where there was an increasing linear deterioration from 10% inclusion of DBR in the diet. The same was reported by Lima et al. (2017), who did not find any worsening of results for any performance variable.

The finding of worsening zootechnical performance as a result of increasing levels of DBR in rabbit diets may be due to some antinutritional factors present in brewery waste, one of which is the hop flower tannin (*Humulus lupulus* L.), which characterizes the bitter taste of beer because it contains bitter resins (Amaro et al., 2009).

Tannins are generally phenolic compounds, soluble in water, and present in the plant kingdom as a form of defense for some plants, being classified as a secondary compound. Therefore, in animal feed, they act by inhibiting the action of some digestive enzymes by complexing with proteins and carbohydrates, reducing the digestibility of the food and also its palatability (Hassan et al., 2020). Since they are considered astringents when in contact with saliva, they form insoluble compounds that lose their lubricity and also inhibit the action of salivary alpha-amylase (Monteiro, 2003), which may explain the decrease in daily feed intake with increasing levels of DBR in the diets.

Not only can tannins act against salivary alpha-amylase, but in the DBR there are other anti-nutritional factors with this function, such as alpha-amylase inhibitors, identified as protein substances that act on starch digestion, interfering with the breakdown of particles and consequently their absorption, due to the inactivation of amylolytic enzymes, and are present in barley and malt (Pagnussatt et al., 2011), ingredients of great importance to the brewing industry. Although the rabbit is a species that is not very dependent on starch in the diet, corn (the main source of starch) was used in the brewing of the pilsen beer that gave rise to the DBR, as well as making up 25% of the experimental diets, with great importance for the digestible energy derived from soluble carbohydrates.

Another antinutritional factor that is very present in barley and therefore in DBR is non-starch polysaccharides, mainly beta-glucans, which are a component of the plant cell wall. According to Furlan et al. (2004), in rabbits, these polysaccharides cause an increase in the viscosity of the digesta, which reduces the rate at which food passes through the intestine so it can cause a reduction in food consumption, as well as a predisposing point for a gradual reduction in feed consumption as a result of increasing levels of residue in rabbit diets. There is also the possibility of the action of phytates and cyanogenic glycosides present in DBR.

There was an effect of DBR on circulating calcium ($P=0.013$) and phosphorus ($P=0.019$) in rabbits, with a linear decrease in the levels of both minerals as a function of increasing levels of waste in the diets (Table 5).

Table 5

Biochemical variables in the blood of rabbits fed isonutritious diets containing increasing levels of dehydrated brewery residue (DBR)

Variables	DBR levels (%)					SEM[1]	P-value
	0	10	20	30	40		
Glucose (mg/dL)	77.41	85.65	80.74	80.37	79.31	2.78	0.211
Total protein (g/dL)	5.81	6.11	5.92	6.34	5.86	0.09	0.474
Albumin (g/dL)	4.10	4.60	4.40	4.28	3.97	0.06	0.163
Globulins (g/dL)	1.71	1.51	1.53	2.06	1.88	0.09	0.512
Urea (mg/dL)	57.20	48.83	48.80	56.33	48.06	1.20	0.178
Calcium (mg/dL)[2]	11.28	11.20	10.06	10.49	8.86	0.21	0.013
Phosphorus (mg/dL)[3]	8.45	9.02	9.37	7.21	7.31	0.20	0.019
Triglycerides (mg/dL)	67.23	97.70	88.62	62.25	97.13	4.98	0.211
Cholesterol (mg/dL)	53.9	62.38	61.67	62.65	61.63	4.17	0.182
HDL (mg/dL)	38.28	29.94	33.20	30.83	29.31	1.99	0.680
LDL (mg/dL)	58.83	75.08	70.35	82.48	82.56	3.50	0.245

[1] Standard error of the mean.
[2] Linear effect (P=0.001): $Y= 11.49 - 0.06x$ ($r^2=0.79$).
[3] Linear effect (P=0.021): $Y= 9.09 - 0.04x$ ($r^2=0.43$).

Despite some antinutritional factors, DBR has a nutrient-rich bromatological composition, with an average of 0.33% Ca and 0.55% total P, values similar to those proposed by the National Research Council [NRC] (1998) for swine, corresponding to 0.32% Ca and 0.56% total P. Nevertheless, these values differ from those presented by Tesser et al. (2022), who reported values of 0.57% Ca and 0.53% P for DBR.

Unlike other mammalian species, rabbits absorb calcium in the intestinal lumen by passive diffusion, which does not necessarily require transcellular transport of vitamin D (Harcourt-Brown, 2002), but they use both methods for its utilization. In terms of calcium metabolism, they do not control intestinal absorption, so if there is an excess in the diet, it is excreted in the urine as calcium carbonate (Meredith & Flecknell, 2006), resulting in thicker, more viscous urine (Harcourt-Brown, 2002). The blood serum of most pets contains approximately 9 to 11 mg/dL of calcium (Vennen & Mitchell, 2009).

In the present study, there was a gradual decrease in plasma levels of Ca and P as a result of the increase in dietary levels of DBR. This decrease may be related to the phytates present in the diet. These compounds are anti-nutritional factors that are also present in brewery waste, especially in the malt used for brewing. They are called secondary compounds and act mainly on the storage of phosphorus by plants, but also on other minerals, making it difficult for the animal organism to use them, especially calcium, which has a strong relationship with phosphorus. In addition to calcium, it can interfere with the absorption of iron, manganese, sodium, and other minerals, as well as the absorption of amino acids, and inhibit the action of trypsin and pepsin (Campestrini et al., 2005).

Phytates reduce the digestibility of the feed and, consequently, the absorption of nutrients by the animal, thus interfering with weight gain. In addition, they have a negative effect on bone and muscle development because calcium is required for the reactions of several endogenous enzymes, so together with the inhibition of pepsin and trypsin, as well as amylase, they reduce protein digestibility and, consequently, muscle deposition. In the present study, this result was observed because as DBR in the diet increased, the animals' daily weight gain and blood calcium and phosphorus levels decreased.

Regarding carcass characteristics, rabbits fed increasing levels of DBR from 31 to 70 days of age showed deterioration with linear reductions ($P<0.05$) in slaughter weight, hot and cold carcass weight, skin yield, head yield, commercial cuts, and relative edible organ weight, as shown in Table 6. Futhermore, the effect of DBR on meat quality was observed, with water loss on thawing ($P=004$) and shear force ($P=0.005$) increasing linearly as a function of increasing levels of residue in the diets, indicating less juiciness and less tenderness in the meat (Table 7).

As a consequence of performance, there have been results of worsening overall carcass characteristics as a result of increasing levels of DBR in the diet, again due to the possibility of anti-nutritional factors. Another hypothesis for the low consumption of feeds containing increasing levels of DBRs is the low palatability of the residue (Frape, 2008), especially for non-ruminants such as rabbits, whose tongues have approximately 17,000 taste buds (Viner, 1999), which could affect the amount of food ingested and the availability of nutrients to the animals.

As the inclusion level of DBR in the diets increased, water loss on thawing and shear force also increased, which may have influenced the calcium levels in the experimental diets on the loss of meat tenderness. However, Lima et al. (2017), did not observe the same results as this study, stating that carcass characteristics were not affected by the addition of the brewery residue.

Meat tenderization is probably the result of several interactive processes, including i) high susceptibility of myofibrils to proteolysis; ii) probable synergism of action of calpains and cathepsins; iii) a large increase in osmotic pressure, possibly causing the release of contractile proteins from myofibrils (Etherington, 1984).

Table 6

Carcass characteristics and organs of rabbits fed diets containing increasing levels of dehydrated brewery residue (DBR)

Variables	DBR levels (%)					SEM[1]	P-value
	0	10	20	30	40		
Live weight at slaughter (g) [2]	2300	2160	2164	2008	1841	35.79	0.005
Hot carcass (g) [3]	1138	1004	1064	946	841	23.25	0.005
Cold carcass (g) [4]	1117	987	1045	930	824	23.11	0.005
Skin, head and cut yield (%)							
Skin [5]	28.59	24.15	26.85	24.51	21.09	0.50	<0.001
Head [6]	17.79	16.19	16.84	16.00	14.43	0.27	0.009
Thoracic-cervical region [7]	24.31	22.46	24.13	22.62	18.78	0.30	0.004
Previous members [8]	15.52	11.36	13.41	12.29	11.34	0.41	0.018
Lumbar region [9]	24.82	20.69	22.60	17.71	17.13	0.77	0.019
Hind limbs [10]	36.19	32.57	33.92	30.66	26.84	0.67	0.002
Sacral region	2.52	2.14	2.16	2.41	1.64	0.11	0.169
Relative weight of organs (%)							
Heart	0.56	0.50	0.57	0.48	0.54	0.01	0.170
Liver [11]	8.25	7.93	6.95	6.68	6.33	0.14	<0.001
Kidneys [12]	1.21	1.03	1.11	1.09	1.07	0.02	<0.001

[1] Standard error of the mean.
[2] Linear effect (P=0.018): Y= 2.308.60 - 10.70x (r^2=0.93).
[3] Linear effect (P=0.033): Y= 1.129.00 - 6.52x (r^2=0.83).
[4] Linear effect (P=0.005); Y= 1.109.20 - 6.43x (r^2= 0.82).
[5] Linear effect (P<0.001); Y= 27.97 - 0.15x (r^2= 0.66).
[6] Linear effect (P=0.020); Y= 17.63 - 0.07x (r^2= 0.78).
[7] Linear effect (P=0.010); Y= 24.64 - 0.11x (r^2= 0.60).
[8] Linear effect (P=0.014); Y= 14.27 - 0.07x (r^2= 0.45).
[9] Linear effect (P<0.001); Y= 24.26 - 0.18x (r^2= 0.80).
[10] Linear effect (P=0.011); Y= 36.16 - 0.21x (r^2= 0.85).
[11] Linear effect (P=0.013); Y= 8.24 - 0.05x (r^2= 0.95).
[12] Linear effect (P=0.033); Y= 1.15 - 0.01x (r^2= 0.67).

Table 7
Meat quality of rabbits fed diets containing increasing levels of dehydrated brewery waste (DRW)

Variables	DBR levels (%)					SEM[1]	P-value
	0	10	20	30	40		
pH 45 min	6.82	6.82	6.90	6.81	6.90	0.03	0.830
pH 24 h	5.59	5.74	5.71	5.62	5.73	0.02	0.111
Minolta L*	64.34	65.18	66.72	65.54	67.87	0.23	0.201
Minolta a*	2.99	3.11	3.48	2.90	3.53	0.12	0.147
Minolta b*	1.15	1.35	1.20	0.95	2.08	0.08	0.101
Cooling water loss (%)	1.89	1.80	1.78	1.79	2.02	0.05	0.605
Thawing water loss (%) [2]	4.21	6.26	8.39	10.08	9.45	0.49	0.004
Cooking water loss (%)	26.37	32.65	30.62	27.93	28.67	0.83	0.195
Shear force (N) [3]	23.11	28.13	28.30	28.51	32.63	1.09	0.005

[1] Standard error of the mean.
[2] Linear effect (P=0.011): Y= 4.82 - 0.13x (r^2=0.87).
[3] Linear effect (P=0.023): Y= 24.25 - 0.19x (r^2=0.83).

Meat tenderness depends on enzymes that act during rigor mortis. These enzymes are known as calpain and calpastatin, which are largely responsible for tenderizing meat and are completely dependent on calcium ions to be activated. Calpastatin acts by inhibiting calpain, which is responsible for the degradation of muscle fibers, and when the calpain-calpastatin complex is formed, calpain is inactivated, thus preventing the degradation of muscle fibers.

According to Machado et al. (2019), the ratio of calcium to phosphorus (Ca:P) for rabbits should be a minimum of 1:1 and a maximum of 2:1. Based on the data presented in Table 2, it can be seen that the total Ca and P levels of all diets are within the commercially recommended limits. According to Andriguetto et al. (2002), an imbalance in either of these micronutrients can ultimately affect the relationship and disrupt the homeostasis process of both components. These minerals are essential for the maintenance of bones and teeth, muscle contraction, blood clotting, membrane permeability, and the nervous system. High calcium levels can cause metabolic disorders such as kidney stones and soft tissue calcification (Vennen & Mitchell, 2009).

Another important point to be highlighted is that there was a similarity between the respective work and the results obtained by Lima et al. (2017) for the variables carcass weight, liver weight and production cost at P<0.05, all of which decreased with the inclusion of brewery residue.

Despite the Ca:P ratio being within the proposed range, it can be seen that the digestibility of calcium in DBR is low (39.88%), as shown in Table 3, which may indicate that the nutrient is absorbed below the actual needs of the body. The digestibility of P is also low (41.15%) and its deficiency results in reduced growth, weight loss, and decreased appetite (De Blas & Mateos, 2010).

In short, although DBR showed significant insoluble fiber levels that could characterize it as a viable alternative food for use in rabbit diets, the performance test showed a decrease in feed consumption and growth, as well as in circulating concentrations of the macrominerals Ca and P, and also in carcass characteristics. Although these results were obtained with a waste product from a craft brewery in Brazil, this type of enterprise is on the rise in the country, which will generate more similar wastes in the short and medium term. In any case, further evaluations are needed in future work, especially focused on assessing possible antinutritional factors, intestinal integrity in rabbits, and gene expression of digestive enzymes.

Conclusion

The DM of dehydrated brewery residue had an average ADC of 64.68 for rabbits, while the ADC of GE and CP were 67.39 and 80.64, respectively, resulting in digestible energy and digestible protein contents of 3,081 and 17.97. Levels of 10 to 40% DBR in feeds for New Zealand White rabbits from 31 to 70 days of age mitigate production costs, but affect performance and reduce circulating levels of calcium and phosphorus, carcass weight, commercial cuts, organs, as well as increase defrosting water loss and reduce meat tenderness.

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