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# INTAKE, DIGESTIBILITY, AND INGESTIVE BEHAVIOR OF HEIFERS FED ALTERNATIVE PROTEIN SOURCES

# CONSUMO, DIGESTIBILIDADE E COMPORTAMENTO INGESTIVO DE NOVILHAS ALIMENTADAS COM FONTES ALTERNATIVAS DE PROTEÍNA

# CONSUMO, DIGESTIBILIDAD Y COMPORTAMIENTO INGESTIVO DE NOVILLAS ALIMENTADAS CON FUENTES ALTERNATIVAS DE PROTEÍNA

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**ABSTRACT**

This study tested the hypothesis that the substitution of up to 20% of the protein source does not affect feed intake, digestibility, or ingestive behavior in dairy heifers. Twenty 15-month-old dairy heifers were allocated to one of four dietary treatments in a completely randomized design. Experimental diets consisted of a corn-soybean meal-based concentrate (control); inclusion of 20% cottonseed cake on a dry matter basis; inclusion of 20% sunflower meal on a dry matter basis; and inclusion of 20% castor cake on a dry matter basis. Dry matter intake and ingestive behavior of the heifers were not affected by diets containing protein sources, only ether extract intake was affected by the treatments, with the highest intake observed in heifers fed a diet containing cottonseed cake. Likewise, nutrient digestibility was not affected by the treatments. Based on the analyzed nutritional parameters, the alternative protein sources-cottonseed cake, sunflower meal, or castor cake-can be included up to 20% of diet dry matter without affecting feed intake, digestibility, or feeding behavior of dairy heifers.

**KEYWORDS:** Castor cake. Cottonseed cake. Sunflower meal.

**RESUMO**

Este estudo testou a hipótese de que a substituição de até 20% da fonte proteica não afeta o consumo de ração, a digestibilidade ou o comportamento ingestivo de novilhas leiteiras. Vinte novilhas, com 15 meses de idade, foram distribuídas em quatro tratamentos dietéticos, em delineamento inteiramente casualizado. As dietas experimentais consistiram em concentrado à base de milho e farelo de soja (controle); inclusão de 20% de torta de algodão na matéria seca; inclusão de 20% de farelo de girassol na matéria seca; e inclusão de 20% de torta de mamona na matéria seca. O consumo de matéria seca e o comportamento ingestivo não foram influenciados pelas dietas com fontes proteicas alternativas; apenas a ingestão de extrato etéreo foi afetada,

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sendo maior nas novilhas alimentadas com dieta contendo torta de algodão. Da mesma forma, a digestibilidade dos nutrientes não diferiu entre os tratamentos. Com base nos parâmetros nutricionais avaliados, as fontes proteicas alternativas – torta de algodão, farelo de girassol e torta de mamona – podem ser incluídas em até 20% da matéria seca da dieta, sem prejuízo ao consumo, à digestibilidade ou ao comportamento ingestivo de novilhas leiteiras.

**PALAVRAS-CHAVE:** Torta de mamona. Torta de algodão. Farelo de girasol.

**RESUMEN**
Este estudio probó la hipótesis de que la sustitución de hasta el 20 % de la fuente proteica no influye en el consumo de alimento, la digestibilidad ni el comportamiento ingestivo de novillas lecheras. Veinte novillas lecheras, con una edad promedio de 15 meses, fueron asignadas a cuatro tratamientos dietéticos en un diseño completamente al azar. Las dietas experimentales estuvieron compuestas por un concentrado a base de maíz y harina de soja (tratamiento control); inclusión del 20 % de torta de algodón sobre base de materia seca; inclusión del 20 % de harina de girasol sobre base de materia seca; e inclusión del 20 % de torta de ricino sobre base de materia seca. El consumo de materia seca y el comportamiento ingestivo de las novillas no se vieron afectados por las dietas que contenían las diferentes fuentes proteicas, sólo la ingesta de extracto de éter se vio afectada por los tratamientos, observándose la mayor ingesta en las novillas alimentadas con una dieta que contenía torta de semilla de algodón. Asimismo, la digestibilidad de los nutrientes no presentó diferencias significativas entre los tratamientos. Con base en los parámetros nutricionales evaluados, las fuentes proteicas alternativas—torta de algodón, harina de girasol y torta de ricino— pueden ser incluidas hasta en un 20 % de la materia seca de la dieta sin comprometer el consumo de alimento, la digestibilidad ni el comportamiento ingestivo de novillas lecheras.

**PALABRAS CLAVE**: Torta de rícino. Torta de algodón. Harina de girasol.

## 1. INTRODUCTION

Brazil has the largest commercial cattle herd in the world, and its production systems are predominantly pasture-based, benefiting from the country's vast territorial extension and favorable climate. However, this almost exclusive reliance on pastures poses significant challenges, such as forage production seasonality and the degradation of grazing areas. These factors limit the continuous supply of nutrients due to annual variations in pasture nutritional quality (De Paula *et al*., 2020; Malafaia *et al*., 2020).

Formulating balanced diets that promote the expression of animals' genetic potential is a fundamental requirement for competitiveness in animal production systems (Paulino *et al.*, 2021; Pereira *et al*., 2023). Therefore, to mitigate the negative effects of pasture seasonality and optimize cattle performance, the use of non-conventional feed sources has emerged as an alternative to reduce this limitation in production systems (Porto *et al*., 2025).

According to Medeiros *et al*., (2009), it is necessary to evaluate new ingredient sources to determine whether their inclusion levels are capable of meeting nutritional requirements and achieving the expected productive performance without increasing concentrate production costs.


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One option that has proven viable is the use of by-products and co-products; however, their adoption must take into account factors such as availability, supply regularity, proximity between the production source and the farm, animal acceptance, nutritional value, cost per kilogram, transportation expenses, and storage conditions, in order to minimize undesirable losses (Lopes *et al*., 2025).

Co-products have potential for use as animal feed, mainly due to their favorable nutritional quality. For instance, cottonseed cake, sunflower meal, and castor cake contain crude protein (CP) levels of 355 g $kg^{-1}$ (Busanello *et al*., 2017), 300 g $kg^{-1}$ DM (Zagorakis *et al*., 2018), and 348 g $kg^{-1}$ DM (Palmieri *et al*., 2016), respectively. Their CP content has enabled the replacement of traditional feedstuffs, such as soybean meal, without compromising animal performance, while reducing feeding costs.

Heifer rearing accounts for up to 20% of total farm costs, while feed expenses represent approximately 50% of total dairy farm expenditures (Costa *et al*., 2007). In Brazil, the search for alternative feed sources has become essential to maintain satisfactory heifer performance at low cost, and the use of agro-industrial co-products can be a viable alternative for developing nutritionally sound feeding strategies that improve heifer productivity while reducing feeding costs (Maciel *et al*., 2012).

The inclusion of brans and cakes in ruminant diets has proven to be a viable alternative feed strategy to reduce the use of corn and soybean meal as dietary ingredients, while ensuring adequate lipid and protein supply to meet animals' nutritional requirements (Lopes *et al*., 2025; Lima *et al*., 2021; Pinto *et al*., 2020). Therefore, the objective of this study was to evaluate the dietary inclusion of three co-products-cottonseed cake, sunflower meal, and castor cake-on feed intake, apparent digestibility, and the feeding behavior of dairy heifers.

## 2. MATERIAL AND METHODS

The experimental protocol followed the ethical principles of animal research and was approved by the Institutional Committee on Animal Use (Protocol 64/2014). The experiment was conducted in Itapetinga, Bahia, Brazil (15°24′75″S, 40°25′10″W).

Twenty Holstein-Zebu heifers were used, with an initial mean body weight of 295 ± 73.7 kg. The animals were housed in individual pens equipped with troughs and automatic water drinkers and were fed diets containing 60% sugarcane and 40% concentrate. The experimental design was completely randomized, consisting of four treatments with five replicates each (4 × 5).

Diets were formulated to provide sufficient nutrients to support a target weight gain of 0.6 kg $day^{-1}$ (NRC, 2001). Sugarcane was supplemented with 1% of a urea + ammonium sulfate (SA) mixture in a 9:1 ratio, based on natural matter. The concentrates used were as follows: standard concentrate based on corn and soybean meal; cottonseed cake: included at 20% of the total diet;


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sunflower meal: included at 20% of the total diet; castor cake: included at 20% of the total diet. Castor cake was detoxified as recommended by Oliveira (2008). The proportions of ingredients and diet compositions are shown in Tables 1 and 2, respectively.

**Table 1.** Chemical composition of ingredients used during experimental period

| Item (%)[1] | Sugar cane +1% urea | Ground corn | Soybean meal | Cottonseed cake | Sunflower meal | Castor cake |
|---|---|---|---|---|---|---|
| Dry matter | 23.2 | 87.5 | 88.5 | 91.5 | 89.5 | 87.0 |
| Crude protein | 9.9 | 6.8 | 45.3 | 35.0 | 32.9 | 34.5 |
| Ether extract | 0.9 | 5.4 | 1.9 | 15.5 | 1.5 | 6.8 |
| aNDF | 51.9 | 16.0 | 9.8 | 43.0 | 44.7 | 41.1 |
| iNDF | 25.8 | 3.1 | 2.1 | 29.5 | 25.1 | 37.0 |
| Acid detergent fiber | 35.1 | 2.6 | 7.3 | 38.2 | 33.3 | 40.4 |
| Total carbohydrates | 86.6 | 86.3 | 47.1 | 57.7 | 66.4 | 50.7 |
| Non-fiber carbohydrates | 34.6 | 70.2 | 46.5 | 15.1 | 22.1 | 11.0 |
| Lignin | 17.6 | 1.2 | 0.8 | 9.8 | 9.4 | 28.0 |
| Ash | 2.6 | 1.5 | 5.8 | 4.0 | 5.6 | 11.5 |
| Total digestible nutrients | 48.0 | 87.5 | 80.3 | 73.7 | 54.9 | 37.8 |

*aNDF*, neutral detergent fiber ash and protein-free; *iNDF*, indigestible neutral detergent fiber.
[1]Nutrient on dry basis (g $kg^{-1}$).
[2]NRC (2001).

**Table 2.** Chemical compositions of the experimental diets

| Item (%)[1] | Standard Concentrate | Diet with Cottonseed cake | Diet with Sunflower meal | Diet with Castor cake |
|---|---|---|---|---|
| Dry matter | 51.9 | 50.2 | 49.9 | 49.3 |
| Crude protein | 13.5 | 13.3 | 13.5 | 13.3 |
| Ether extract | 3.3 | 3.8 | 2.8 | 2.7 |
| aNDF | 39.4 | 45.5 | 40.7 | 40.1 |
| iNDF | 17.0 | 21.8 | 26.7 | 24.9 |
| Acid detergent fiber | 23.1 | 34.3 | 27.7 | 30.1 |
| Total carbohydrates | 78.2 | 77.5 | 77.8 | 76.6 |
| Non-fiber carbohydrates | 38.9 | 31.9 | 37.1 | 36.5 |
| Lignin | 10.7 | 14.4 | 12.3 | 17.0 |
| Ash | 4.9 | 5.5 | 5.9 | 7.4 |
| Total digestible nutrients | 62.1 | 49.6 | 54.1 | 56.2 |

*aNDF*, neutral detergent fiber ash and protein-free; *iNDF*, indigestible neutral detergent fiber.
[1]Nutrient on dry basis (g $kg^{-1}$).
[2] NRC (2001).

The presence of ricin in the sample was confirmed using capillary electrophoresis and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the Hoefer minigel system.


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The electrophoresis analysis showed that the ricin content in the sample detoxified using the process proposed in this study was reduced following treatment with $Ca(OH)_2$ solution. The detoxification of the castor cake was considered effective when no visible bands corresponding to standard ricin were observed in the gels (Figure 1).

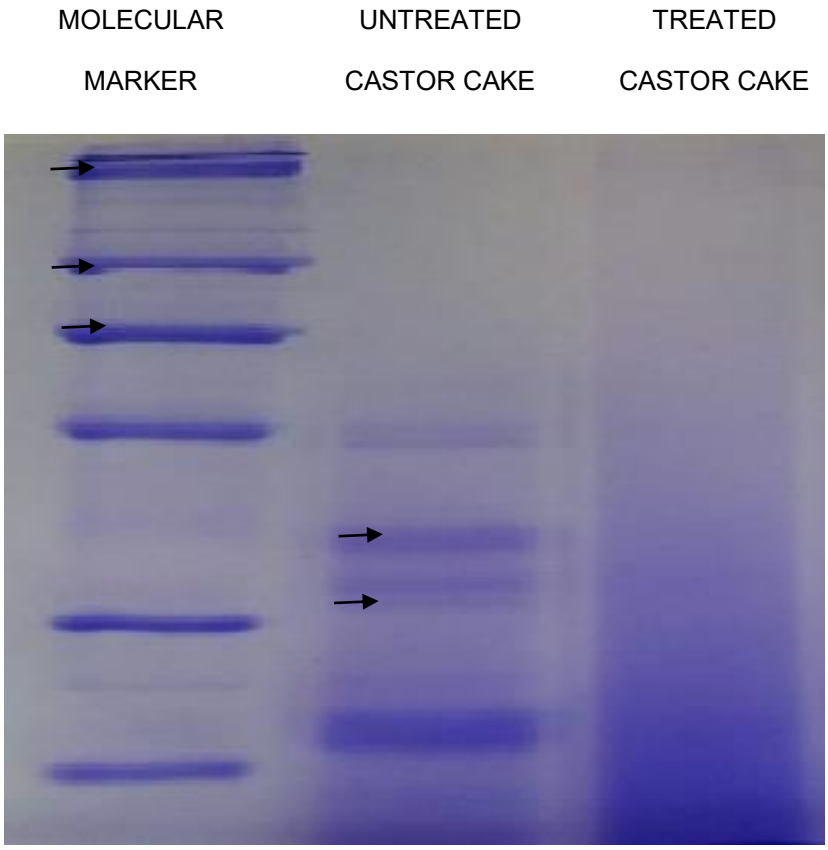



**Figure 1.** Polyacrylamide gel electrophoresis of castor cake treated or untreated with $Ca(OH)_2$.

The chemical composition of sugarcane, corn, soybean meal, cottonseed cake, sunflower meal, and castor cake is presented in Table 1, and the composition of the diets is shown in Table 2.

The experiment lasted 19 days, including 14 days for animal adaptation and 5 days for data collection. Diets were provided ad libitum twice daily, with refusals ranging from approximately 5 to 10% of the supply, and water was available to the animals at all times. Feed offered was recorded daily throughout the experiment. Diet samples and refusals were stored at -20 °C for further analysis. Animals were weighed at the beginning and end of the experiment to estimate nutrient intake as a percentage of body weight.

Samples of diets, concentrated ingredients, refusals, and feces were analyzed to evaluate feed intake and digestibility. Analyses of dry matter (DM; method 967.03), ash (method 942.05),


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crude protein (CP; method 981.10), and ether extract (EE; method 920.39) were performed according to AOAC (1990). Neutral detergent fiber, ash- and protein-free (aNDF) was determined using sodium sulfite-free thermostable alpha-amylase (Mertens, 2002; Licitra *et al*., 1996), and acid detergent fiber (ADF) was determined as described by Van Soest *et al*., (1991). Lignin content was determined by treating the ADF residue with 72% sulfuric acid (Detmann *et al*., 2012). Non-fibrous carbohydrate (NFC) content was calculated as described by Detmann *et al.,* (2012) using the equation: NFC = 100 – ash – EE – aNDF – CP.

To estimate apparent nutrient digestibility, feces were collected from the animals for two consecutive days at different times (12:00 and 17:00) on the 18th and 19th days of the experiment. Indigestible neutral detergent fiber (iNDF) was used as an internal marker to estimate fecal excretion (Detmann *et al*., 2001; Detmann *et al*., 2007). Fecal output was calculated based on the relationship between iNDF intake and its concentration in feces.

Animal feeding behavior was evaluated during the last two days of the experimental period by recording activities at five-minute intervals over a 48-hour period. The time spent eating, ruminating, and idle was observed (Mezzalira *et al*., 2011). Nocturnal observations were conducted under artificial lighting. During the 48-hour period, observations were made in the morning, at noon, and in the evening by trained personnel using a digital timer. Behavioral variables related to feeding (eating, ruminating, and idle times) were recorded according to Carvalho *et al*. (2011). The number of chewed ruminal boluses and the time spent ruminating each bolus were also recorded. The daily number of boluses ruminated was calculated by dividing the total rumination time (min) by the average time spent ruminating a single bolus.

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) according to the following statistical model:

$$Y_{ijk} = \mu + D_i + e_{ijk}$$

Where: $Y_{ijk}$ = dependent variable; $\mu$ = overall mean; $D_i$ = Diet; = $e_{ijk}$ = Residual error.

Diet effect were analyzed as a fixed effect for all measurements. Tukey test was used as a means comparison with significance declared when $P \leq 0.05$.

## 3. RESULTS AND DISCUSSION

There was no effect of replacing corn and soybean meal with co-products on most variables (expressed in kg day$^{-1}$, as a percentage of body weight - %BW, or as metabolic weight - g/kg$^{0.75}$). Only ether extract intake (kg day$^{-1}$) was affected by the treatments (P = 0.003), with the highest intake (0.34 kg day$^{-1}$) observed in heifers fed a diet containing cottonseed cake. On average, the heifers consumed 9.01 kg of DM, 1.15 kg of CP, 3.38 kg of aNDF, and 5.92 kg of

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TDN. The mean intakes of DM, CP, and aNDF as a percentage of body weight were 2.90, 0.38, and 1.08%, respectively (Table 3).

**Table 3.** Intakes of dry matter (DM), and nutrients by dairy heifers fed diets containing co-products as alternative protein sources

| Item | Treatment | | | | SE[1] | P-value |
|---|---|---|---|---|---|---|
| | Standard Concentrate | Diet with Cottonseed cake | Diet with Sunflower meal | Diet with Castor cake | | |
| | | | Intake (kg day$^{-1}$) | | | |
| DM | 9.29 | 9.13 | 9.11 | 8.52 | 0.093 | 0.821 |
| CP | 1.21 | 1.16 | 1.16 | 1.05 | 0.002 | 0.648 |
| EE | 0.32ab | 0.34a | 0.25bc | 0.22c | <0.001 | 0.003 |
| iNDF | 5.44 | 5.87 | 6.43 | 5.60 | 0.047 | 0.429 |
| aNDF | 3.25 | 3.83 | 3.31 | 3.12 | 0.016 | 0.272 |
| NFC | 3.04 | 2.88 | 3.08 | 2.53 | 0.012 | 0.315 |
| TDN | 6.04 | 6.45 | 5.81 | 5.37 | 0.047 | 0.396 |
| | | | Intake (% body weight) | | | |
| DM | 3.0 | 3.0 | 2.9 | 2.7 | 0.006 | 0.469 |
| CP | 0.4 | 0.4 | 0.4 | 0.3 | <0.001 | 0.286 |
| aNDF | 1.1 | 1.2 | 1.0 | 1.0 | 0.001 | 0.063 |
| TDN | 2.0 | 2.1 | 1.8 | 1.7 | 0.004 | 0.246 |
| | | | Intake (metabolic weight g/kg$^{0.75}$) | | | |
| DM | 126.6 | 124.1 | 122.1 | 113.6 | 9.168 | 0.487 |
| CP | 16.5 | 15.7 | 15.6 | 14.0 | 0.202 | 0.308 |

*CP*, crude protein; *EE,* ether extract; *iNDF*, indigestible neutral detergent fiber; *aNDF*, neutral detergent fiber ash and protein-free; *NFC*, non-fiber carbohydrates; *TDN*, total digestible nutrients. [abc]means within a row without common superscripts are different at P<0.05; [1]*SE*, standard error of the mean.

Overall, the use of co-products in animal feed has yielded satisfactory results due to their similarity to traditional feedstuffs in chemical composition (Table 1). Depending on the availability of the co-product, feed costs can be significantly reduced without affecting animal performance (Erickson *et al*., 2012 ; Popp *et al*., 2016). For co-products derived from castor cake, treatment with $Ca(OH)_2$ is essential for detoxification (Figure 1).

Dry matter intake is considered one of the main factors correlated with ruminant production, as dry matter contains the nutrients necessary to meet the requirements of the animal. In our study, the mean DM intake was 9.01 kg day$^{-1}$, corresponding to 2.9% of body weight. The value estimated by Valadares Filho *et al*., (2016) for crossbred dairy cows weighing 295 kg was


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6.03 kg day$^{-1}$, representing 2% BW. The higher feed intake observed in this study can be explained by the higher proportion of Holstein genes in the heifers.

The aNDF intake ranged from 1.0 to 1.2% BW, which is similar to the value of 1.2% BW reported by Mertens (1992), who described NDF intake as one of the main regulatory mechanisms of dry matter intake. The higher DM intake observed indicates that the NDF content was not limiting, which is also a consequence of the diet composition (Table 1).

The mean TDN intake of 5.92 kg day$^{-1}$ was also higher than the value estimated by Valadares Filho *et al*., (2016) of 3.83 kg day$^{-1}$. Similarly, Garcia *et al*., (2006) found no differences in DM intake in growing dairy heifers fed sunflower meal at levels of 0%, 15%, 30%, and 45% as a replacement for soybean meal. On the other hand, Domingues *et al*. (2010) observed that the inclusion of sunflower cake as a replacement for cottonseed meal led to decreased DM intake in feedlot cattle. According to these authors, the high ether extract content in sunflower cake may be responsible for the decrease in DM intake. In the present study, sunflower was fed in the form of meal, which has a lower ether extract content due to the higher efficiency of the extraction method used.

Matos *et al*., (2018) replaced soybean meal with castor seed meal up to 100% in a supplement for grazing heifers and observed that using up to 100% castor meal reduced DM intake without affecting average daily gain or feed efficiency. According to the authors, the decrease in DM and nutrient intake is related to lower forage intake, as well as decreased DM digestibility with increasing levels of castor meal in the diet. In the present experiment, the inclusion of 20 g kg$^{-1}$ of diet DM did not affect DM or nutrient intake in confined heifers. Likewise, Mota *et al*., (2013) studied the effects of different protein sources-soybean meal, cottonseed meal, and sunflower meal-on DM and nutrient intake of Holstein × Zebu dairy heifers and reported no effect of these protein sources on intake.

The apparent digestibility of DM and nutrients was not influenced by the dietary inclusion of co-products, with mean values of 70.78, 78.18, and 54.35% for DM, CP, and NDF, respectively (Table 4).


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**Table 4.** Coefficients of digestibility in dairy heifers fed diets containing co-products alternative protein sources

| Item | Treatment | | | | SE[1] | P-value |
|---|---|---|---|---|---|---|
| | Standard Concentrate | Diet with Cottonseed cake | Diet with Sunflower meal | Diet with Castor cake | | |
| DM | 72.5 | 71.4 | 68.6 | 70.6 | 1.857 | 0.776 |
| CP | 74.7 | 76.6 | 79.2 | 82.2 | 0.929 | 0.081 |
| EE | 82.7 | 87.2 | 84.3 | 89.1 | 0.849 | 0.382 |
| aNDF | 56.9 | 59.4 | 47.8 | 53.3 | 4.649 | 0.304 |
| NFC | 83.6 | 85.5 | 83.9 | 79.6 | 1.214 | 0.323 |
| TDN | 67.0 | 63.4 | 64.4 | 67.0 | 2.057 | 0.755 |

*DM*, dry matter; *CP*, crude protein; *EE*, ether extract; *aNDF*, neutral detergent fiber ash and protein-free; *NFC*, non-fiber carbohydrates; *TDN*, total digestible nutrients.
[1]*SE*, standard error of the mean.

Similarly, DM digestibility was not affected by different protein sources, averaging 70.7% across all treatments. Nutrient digestibility did not differ between treatments (Table 4). It should be emphasized that several factors can influence these parameters, including the composition of ingredients and diets, associative effects between feedstuffs, feeding frequency, rates of degradability and passage, protein-to-energy ratio, and intrinsic animal factors (Van Soest, 1994 ; Orskov, 2000).

The digestibility values indicate that the co-products studied have high total digestibility, comparable to that of corn and soybean meal. Goes *et al*., (2019) evaluated the use of sunflower cake as a replacement for soybean meal on in vitro digestibility in beef cattle. They concluded that sunflower cake could replace up to 280 g $kg^{-1}$ of soybean meal in the concentrate, improving the in vitro digestibility of dry matter, organic matter, and crude protein. The potential of co-products in ruminant feed was also evaluated by Silva *et al*., (2015) using the in vitro gas production technique; these authors reported that cottonseed and sunflower meals have great potential to substitute soybean meal in ruminant diets.

The variables related to feeding behavior, including feeding and rumination efficiencies, the number of feeding, rumination, and resting periods, as well as the time spent feeding, ruminating, and resting, did not differ between heifers fed diets containing different co-products and control heifers. On average, heifers spent 5.5 hours feeding, 8.45 hours ruminating, and 10.05 hours resting (Table 5).


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**Table 5.** Feeding behavior of dairy heifers fed diets containing co-products alternative protein sources

| Item | Treatment | | | | SE[1] | P-value |
|---|---|---|---|---|---|---|
| | Standard Concentrate | Diet with Cottonseed cake | Diet with Sunflower meal | Diet with Castor cake | | |
| | Intake in 24 hours (k day$^{-1}$) | | | | | |
| DM | 8.66 | 8.39 | 8.34 | 7.68 | 0.093 | 0.821 |
| aNDF | 3.72 | 4.26 | 3.84 | 3.64 | 0.020 | 0.444 |
| | (h day$^{-1}$ ) | | | | | |
| Feeding | 5.7 | 5.8 | 5.0 | 5.7 | 0.016 | 0.697 |
| Rumination | 8.4 | 8.4 | 8.6 | 8.4 | 0.050 | 0.066 |
| Idleness | 9.9 | 9.8 | 10.6 | 9.9 | 0.052 | 0.686 |
| | Chewing | | | | | |
| bolus (n day$^{-1}$) | 563.8 | 506.6 | 540.6 | 495.7 | 374.310 | 0.653 |
| n day$^{-1}$ | 30544 | 33810 | 34079 | 29810 | 798.287 | 0.992 |
| h day$^{-1}$ | 14.0 | 14.2 | 13.4 | 14.1 | 0.052 | 0.686 |
| n bolus$^{-1}$ | 54.8 | 66.8 | 63.8 | 60.3 | 3.631 | 0.838 |
| s bolus$^{-1}$ | 53.8 | 59.6 | 58.7 | 61.0 | 4.072 | 0.600 |

*DM*, dry matter; *aNDF*, neutral detergent fiber ash and protein-free; *bolus (n day$^{-1}$)*, number of bolus chewing per day; *n day$^{-1}$*, number of chewing per day; *h day$^{-1}$*, hours of chewing per day; *n bolus$^{-1}$*, number of chews per day; *s bolus$^{-1}$*, time in seconds for chewed bolus.
[1]*SE*, standard error of the mean.

The dietary inclusion of co-products did not affect the feeding behavior of heifers (Table 5). Thus, the intakes of dry matter and ash- and protein-free neutral detergent fiber, in kg day$^{-1}$, the time spent feeding, ruminating and resting, expressed in h day$^{-1}$, were similar among heifers regardless of the diets. It demonstrates that the intake was regulated by the fill effect of fiber since the proportion of roughage used in the diets has led to high fiber intake. The number of ruminal boluses, number of chews, time spent chewing, chewing number per bolus and chewing time per bolus (sec$^{-1}$) were not influenced by diets (Table 5) and may be associated with the similarity between the chemical composition of diets (Table 2).

Regarding feed efficiency, the animals consumed, on average, 1.51 kg of DM h$^{-1}$ and 0.70 kg of aNDF h$^{-1}$, and ruminated 0.985 kg of DM h$^{-1}$ and 0.46 kg of aNDF h$^{-1}$ (Table 6). On average, the number of feeding periods was 17.6, with a mean duration of 19.15 minutes each, whereas the number of rumination periods was 18.35, with an average duration of 27.8 minutes. Additionally, the number of resting periods per day was 29.4, with a mean duration of 20.75 minutes each (Table 7).


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**Table 6.** Feed efficiency and Rumination of dairy heifers fed diets containing co-products alternative protein sources

| Item | Treatment | | | | SE[1] | P-value |
|---|---|---|---|---|---|---|
| | Standard Concentrate | Diet with Cottonseed cake | Diet with Sunflower meal | Diet with Castor cake | | |
| Feed efficiency | | | | | | |
| kg DM $h^{-1}$ | 1.52 | 1.46 | 1.74 | 1.35 | 0.075 | 0.714 |
| kg aNDF $h^{-1}$ | 0.65 | 0.74 | 0.80 | 0.64 | 0.001 | 0.222 |
| Feed Rumination | | | | | | |
| kg DM $h^{-1}$ | 1.03 | 1.01 | 0.98 | 0.92 | 0.029 | 0.460 |
| kg aNDF $h^{-1}$ | 0.44 | 0.51 | 0.45 | 0.44 | <0.001 | 0.394 |

*DM*, dry matter; *aNDF*, neutral detergent fiber ash and protein-free. [1]*SE*, standard error of the mean.

**Table 7.** Number of periods and average time spent per period on the feeding, ruminating, and idle activities by dairy heifers fed diets containing co-products alternative protein sources

| Item | Treatment | | | | SE[1] | P-value |
|---|---|---|---|---|---|---|
| | Standard Concentrate | Diet with Cottonseed cake | Diet with Sunflower meal | Diet with Castor cake | | |
| Number of periods (n $d^{-1}$) | | | | | | |
| Feeding | 18.5 | 17.9 | 15.4 | 18.5 | 0.356 | 0.554 |
| Ruminating | 17.4 | 19.0 | 18.0 | 19.0 | 0.248 | 0.644 |
| Idling | 29.9 | 31.0 | 28.5 | 28.2 | 0.538 | 0.792 |
| Time spent per period (min) | | | | | | |
| Feeding | 18.9 | 20.1 | 18.9 | 18.7 | 0.764 | 0.138 |
| Ruminating | 29.4 | 26.6 | 28.9 | 26.3 | 1.065 | 0.608 |
| Idling | 20.3 | 19.0 | 22.4 | 21.3 | 0.429 | 0.212 |

[1]*SE*, standard error of the mean.

According to Grant and Ferraretto (2018), the chewing index decreases under the following conditions: increased NDF digestibility, decreased particle size, or reduced NDF content. As previously mentioned, none of these conditions were observed in the present study. Feeding and rumination efficiencies are primarily affected by feed intake (Carvalho *et al*., 2011); therefore, since there was no difference in dry matter intake, no change was observed in the amount of feed chewed (on a DM basis) per hour. In the present study, the equivalence in feeding and rumination efficiencies was likely due to the similarity in time spent on these activities (Table 6).


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The number of feeding, rumination, and resting periods, as well as the time spent feeding, ruminating, and resting, did not differ significantly (Table 7). According to Carvalho *et al*. (2017), the feeding frequency of twice daily may explain the lack of significant differences.

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## 5. CONCLUSION

Based on the analyzed nutritional parameters, the alternative protein sources cottonseed cake, sunflower meal, and castor cake can be included at up to 20% of diet dry matter without affecting feed intake, nutrient digestibility, or feeding behavior in dairy heifers.

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