By-Product of Cotton Agribusiness as an Alternative Protein Source for Rams

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Received: 26 May 2020; Accepted: 3 July 2020; Published: 8 July 2020

Abstract: The aim of this study was to evaluate the effect on intake, digestibility and fractionation of carbohydrates and proteins of cottonseed cake as an alternative protein source in rams diets. The diets were composed of corn silage, soybean meal, ground corn, urea, and cottonseed cake (0, 70, 140 and 210 g/kg dry matter (DM)) in a roughage/concentrate ratio of 50:50. Thirty-two Santa Inês rams, average age 12 months and weight 27.48 ± 4.96 kg were distributed in a completely randomised design. A reduction of 0.161 g/day in crude protein (CP) intake was observed for each gram of cottonseed cake inclusion. The intake of non-fiber carbohydrate (NFC) decreased by 0.511 g/day for each gram of cottonseed cake inclusion. Similarly, the ratio between intake of DM (p < 0.001), CP (p < 0.001), NFC (p = 0.004) and body weight decreased with cottonseed cake inclusion. Inclusion of cottonseed cake caused decreases in the digestibility coefficients of DM (p < 0.001), CP (p < 0.001) and NFC (p = 0.002). A reduction of 0.716 g was observed in carbohydrate fraction A + B1 (p = 0.006). The inclusion of cottonseed cake in the rams feed should be carried out with caution because this by-product promotes linear reduction in intake and digestibility nutrient, in addition to modifying the amounts of digestible and indigestible fractions in the diets.

Keywords: alternative feed; digestibility; intake; ovine; sustainability

1. Introduction

The confinement system provides the farmer with better animal performance and production than pasture production systems, but the feed traditionally used to feed the animals in confinement system, such as ground corn and soybean meal, has a high cost. Therefore, alternative, cheaper feeds, available in the region and that present good nutritional quality are recommended. In this way, cottonseed cake, which is a by-product of agroindustry, is an alternative feed for ruminants. It is cheaper than those ingredients commonly used in animal feed, and has a suitable chemical composition and great potential for use in the ruminant nutrition [1–3].
In Brazil, most of the agroindustry by-products generated by the extraction of oil and fruit juice, cereal processing and industrialization can be used in animal feed. By-products can reduce diets costs, since cottonseed cake costs about 40% that of soybean meal, to the farmer. Providing feed, with low-cost and alternative feed quality to meet animal’s nutritional requirements without competing with food for human’s, ensures a more sustainable production system [4–7].

Among these by-products that have great potential for use in the ruminant diets formulation, cottonseed cake has, on average, 89.1–94.2% dry matter (DM); 25.9–47.6% crude protein (CP); 1.2–11.4% ether extract (EE); 28.4–33.1% neutral detergent fiber (NDF) and 4.4–15.1% lignin [8,9]. Some authors report that cottonseed cake contains gossypol, a potentially toxic substance that can negatively affect the liver, kidneys, immune system and mainly the reproduction system [10]. However, the literature shows that gossypol is present in a higher concentration in cottonseed oil than in the cake. Therefore, the by-products from the extraction of cottonseed oil contain lower concentrations of gossypol and when present in diets at a concentration of less than 180 mg/day do not cause harmful effects in animals [10,11]. The cottonseed cake has 430 mg/kg DM of gossypol, thus the aforementioned amount of gossypol in the diet represents the use of an amount of approximately of 400 g of cottonseed cake [10–12].

Therefore, knowledge on sustainability feeding becomes of extreme importance for the formulation of ruminant diets to guarantee the animal its best performance. Converting a by-product into animal protein of high biological value for human intake is a worthwhile objective.

In view of the above, this study aimed to evaluate the replacement of soybean meal with cottonseed cake in the diet of feedlot rams and to obtain information related to the intake, digestibility and fractionation of carbohydrates and proteins.

2. Materials and Methods

The experiment was performed from August to October 2014 in the animal performance shed of the Sheep Sector of the Animal Science Faculty of the Federal University of Mato Grosso, Campus of Rondonópolis, MT, Brazil, (16°28’ S; 50°34’ W) according to the recommendations of the Federal University of Mato Grosso Animal Use and Care Committee (Protocol Number 23108.046399/13-4).

2.1. Location, Animals and Diets

The climate is Aw according to the Köppen classification: Tropical with well-defined dry and rainy seasons, hot and humid summers and cold and dry winters. The relative humidity is 60% and the average rainfall is 1240 mm. The annual average temperature is 27.5 °C, ranging between 17 and 38 °C minimum and maximum averages, respectively. The trial was performed during August to October of 2014; in this period the average temperature was 25 °C and the total rainfall was 200 mm.

A total of 32 rams of the Santa Ines breed, average age 12 months and average initial weight 27.48 ± 4.96 kg, were distributed in a completely randomised block design with eight replicates. The animals previously consumed a diet with 50:50 roughage:concentrate and because of this they were subjected to a period of seven days to adaptation period to the inclusion of cottonseed cake in the diets, environment and management, with the total experimental period of 60 days evaluation.

The animals were allocated in separate and individual pens of 2.5 m² equipped with feeders, salt troughs and drinking fountains. After weighing, identification and treatment against ecto- and endoparasites, the animals were vaccinated against clostridial disease. The treatments consisted of the replacement of 0, 70, 140 and 210 g/kg soybean meal by cottonseed cake in the dry matter (DM) of concentrated feed. The diets with forage (50%) and concentrate (50%) on a DM basis composed of corn silage, soybean meal, cottonseed cake, ground corn and urea (Table 1) were formulated according to the requirements for late-maturing sheep of 30 kg body weight and estimated weight gain of 0.200 kg/day and prepared to contain 133 g/kg of CP and 570 g/kg of total digestible nutrient (TDN) [13].
Table 1. Chemical composition of corn silage (CS), ground corn (GC), soybean meal (SM) and cottonseed cake (CC).

| Component (g/kg DM)          | Ingredient (g/kg) |
|-----------------------------|-------------------|
|                             | CS        | GC       | SM       | CC       |
| Dry matter (g/kg Natural matter) | 297.0    | 908.5    | 911.6    | 940.0    |
| Mineral matter              | 41.2     | 12.0     | 70.7     | 42.5     |
| Crude protein               | 53.7     | 82.4     | 450.1    | 302.0    |
| Ether extract               | 28.6     | 53.9     | 12.4     | 100.0    |
| Total carbohydrate          | 876.5    | 851.7    | 466.8    | 555.5    |
| Acid detergent fiber        | 253.6    | 30.3     | 96.0     | 364.9    |
| NDFap a                     | 451.1    | 108.2    | 102.4    | 473.3    |
| Non-fibrous carbohydrates   | 425.4    | 743.5    | 364.4    | 82.2     |
| iNDF b                      | 217.3    | 14.9     | 16.0     | 270.5    |
| Hemicellulose               | 197.5    | 77.9     | 6.4      | 88.4     |
| Total nitrogen              | 8.59     | 13.2     | 72.0     | 48.3     |
| NDFIN c                     | 166.80   | 102.40   | 31.70    | 171.10   |
| ADIN d                      | 74.70    | 40.30    | 17.90    | 102.00   |

| Fractional composition of carbohydrates (g/kg of total carbohydrate) |
|-------------------------------------------------------------|
| Fraction A + B_1                                            | 284.9  | 867.6  | 735.8  | 395.3  |
| Fraction B_2                                                | 515.4  | 97.79  | 173.2  | 319.3  |
| Fraction C                                                  | 199.7  | 34.57  | 90.99  | 285.4  |

| Fractional composition of proteins (g/kg of total nitrogen) |
|-----------------------------------------------------------|
| Fraction A                                                 | 337.1  | 173.8  | 187.2  | 226.3  |
| Fraction B_1 + B_2                                         | 414.4  | 750.9  | 754.7  | 693.8  |
| Fraction B_3                                                | 135.1  | 54.77  | 32.04  | 28.41  |
| Fraction C                                                  | 123.4  | 20.53  | 26.06  | 51.49  |

* NDFap = NDF corrected for ash and protein; b iNDF = Indigestible NDF; c NDFIN = Neutral detergent insoluble nitrogen; d ADIN = Acid detergent insoluble nitrogen.

The diets (Table 2) were provided as a total mixed ration twice per day at pre-established times of 7:30 a.m. and 3:30 p.m. The amounts of the daily diet supplied per animal were adjusted, in order to allow 20% of leftovers on natural matter basis. Mineral salt was offered ad libitum to the animals; the mineral salt composition is shown at the bottom of Table 2.

The NDF residue was corrected for ash and protein (NDFap) according to the methodologies proposed by [14]. The iNDF of the feed was obtained through in-situ incubation for 240 h [15], followed by NDF analysis.

The digestibility assay was performed between the 31st and 34th days of the experiment, for this daily samples of the supplied feed, leftovers and feces were obtained, which were frozen and subsequently homogenized, in order to obtain the respective composite samples. These composite samples were also processed as described below.

Samples of ingredients and leftovers, of the whole experimental trial, were pre-dried in a forced-ventilation oven at 55 °C for 72 h and ground in a Wiley knife mill (Wiley mill, Arthur H. Thomas, PA, USA) using 1 mm sieves for subsequent analyses of dry matter (DM, method 934.01), ashes (method 924.05), crude protein (CP, N × 6.25; method 976.06), ether extract (EE, method 945.16) and acid detergent fiber (ADF, method 973.18) according to [16]. NDF and ADF were determined using the methods of [17], previously treated with heat-stable alpha-amylase [18]. The NDF corrections for ashes and protein (NDFap) were performed according to [19]. The total carbohydrates (TCs) were calculated according to [20]: \[ TC = 100 - \left(\% CP + \% Ash + \% EE\right). \] Due to the presence of urea in the diets, the non-fibrous carbohydrates (NFCs) were calculated according to the equation proposed by [21]: \[ NFC = 100 - \left(\% CP - \% CP derived from urea + \% urea\right) + \% NDFap + \% EE + \% Ash. \]

To obtain the total calculated TDN content, the following equation was used: \[ \% TDNc = 3.71095 - 0.129014 \times NDF + 1.02278 \times OMD \] [22].
Table 2. Ingredient proportions and chemical composition of the experimental diets to rams containing different levels of cottonseed cake.

| Ingredient                  | Cottonseed Cake Content (g/kg DM) |
|-----------------------------|-----------------------------------|
|                             | 0       | 70      | 140     | 210     |
| Corn silage                 | 500     | 500     | 500     | 500     |
| Ground corn                 | 290     | 288     | 284     | 280     |
| Soybean meal                | 210     | 140     | 70      | 0       |
| Cottonseed cake             | 0.0     | 70      | 140     | 210     |
| Urea                        | 0.0     | 2.0     | 6.0     | 10.0    |
| Dry matter (g/kg Natural Matter) | 603.4   | 605.6   | 607.9   | 610.2   |
| Mineral matter              | 38.9    | 36.9    | 34.9    | 32.9    |
| Crude protein               | 142.3   | 138.4   | 134.9   | 131.5   |
| Ether extract               | 32.5    | 38.6    | 44.5    | 50.4    |
| Total carbohydrates         | 783.3   | 787.8   | 790.6   | 793.4   |
| Acid detergent fiber        | 155.7   | 175.9   | 196.0   | 216.1   |
| NDFap a                     | 278.4   | 304.2   | 329.7   | 355.2   |
| Non-fibrous carbohydrates   | 504.8   | 483.6   | 460.9   | 438.1   |
| iNDF b                      | 116.3   | 134.1   | 151.9   | 169.6   |
| Hemicellulose               | 122.7   | 128.3   | 133.7   | 139.1   |
| Total nitrogen              | 22.8    | 22.1    | 21.6    | 21.0    |
| NDIN c (g/kg Total nitrogen)| 119.8   | 129.3   | 138.7   | 148.0   |
| ADIN d (g/kg Total nitrogen)| 52.8    | 58.6    | 64.3    | 70.1    |
| TDNc e                      | 655.7   | 640.3   | 607.8   | 583.4   |

Cost of total mixed ration (US $/kg) * 0.10 0.09 0.08 0.06

* NDFap = NDF corrected for ash and protein; iNDF = Indigestible NDF; NDIN = Neutral detergent insoluble nitrogen; ADIN = Acid detergent insoluble nitrogen; TDNc = Calculated total digestible nutrients. Mineral salt mixture provided ad libitum—Guaranteed levels (g/kg DM): calcium 17.7, phosphorus 8.0, sodium 0.4, sulfur 2.0, copper 0.55, iodine 6.0, manganese 12.0, selenium 0.15, zinc 3.0, and fluoride 8.0 maximum. * Values estimated in the Brazilian market in the June 2020.

2.2. Feed Intake, Nutrient Digestibility and Animal Performance

To estimate the dry matter intake (DMI), the offered feed and leftovers per animal were weighed daily. The total offered feed and leftovers were divided by the number of days in the feedlot (60 days), resulting in the daily averages of offered feed and refusals. The intakes of DM, CP, organic matter (OM), EE, NDF, NFC, TC, TDNc and indigestible neutral detergent fiber (iNDF) were expressed as g/animal/day as percentages of body weight (% BW) and as g/kg of metabolic weight (g/kg BW0.75).

The total digestible nutrient intake (TDNI) of each animal was estimated by the difference between the amounts of each nutrient ingested and recovered in the feces, according to the equation: TDNI (kg/day) = (CPi − CPf) + 2.25 (EEi − EEf) + (NFCi − NFCf) + (FCi − FCI) [21], where the indices i and f correspond to the element ingested and excreted in feces, respectively.

The water intake of each animal was quantified by the daily difference between the supplied volume and the leftover water, using graduated buckets. Together with the water supply, two buckets of water were placed in the installation, in order to measure evaporation losses [23].

The digestibility coefficients of DM, CP, OM, EE, NDF, NFC and TC were determined using the total feces, collected in individual collector bags tied to the animals.

The feces were collected manually twice a day, always 1 h prior to the morning and afternoon feeding. Immediately after each weighing, fecal samples were taken per animal and frozen in individual plastic bags.

All the samples from to each animal were thawed, mixed and homogenised and aliquots taken to form composite samples, which were pre-dried in a convection oven at 60 °C for approximately 72 h and then ground in a Thomas Wiley mill with 1 mm sieves.
The DM and nutrient digestibility coefficients (DC) of the diets were calculated based on the difference between the amounts ingested and excreted in the feces of each nutrient, using the formula: 

$$DC = \left( \frac{\text{nutrient ingested} - \text{nutrient in feces}}{\text{ingested nutrient}} \right) \times 100.$$ 

2.3. Fractionation of Carbohydrates and Proteins

The fractions A + B₁, B₂ and C of carbohydrates were determined to represent soluble sugars, starch and pectin, the potentially digestible NDF and the iNDF, respectively. The TCs were calculated based on the equation: 

$$TC = 100 - (\%CP + \%MM + \%EE)$$  

The fraction C, corresponding to iNDF, was determined after 144 h of in-situ incubation [15,24]. The fraction B₂, which corresponded to the available fraction of the fiber, was obtained by the difference between the NDFap and the fraction C.

Protein fractions (A, B₁ + B₂, B₃ and C) were determined, with fraction A corresponding to non-protein nitrogen (NPN), fraction B₁ to true protein from rapid degradation in the rumen (>50%/h), fraction B₂ of intermediate degradation rate (5-15%/h), fraction B₃ of slow degradation rate (0.01–1.5%/h), and fraction C corresponded to acid detergent insoluble nitrogen (ADIN). In the present study, the fractions B₁ and B₂ were considered as a single fraction (B₁ + B₂) and refer to insoluble and potential degradable proteins. The fractions B₁ + B₂ were obtained by the difference between fraction A and the neutral detergent insoluble nitrogen (NDIN) content [19]. The fraction B₃ was obtained by the difference between NDIN and ADIN.

The NPN content (fraction A) was calculated as the difference between the percentage of total nitrogen in the sample and the value of protein nitrogen contained in the residue after sample treatment with 10% trichloroacetic acid solution for 30 min. Then, the nitrogen content in the residue was estimated by the macro Kjeldahl [14,22]. The NDIN and ADIN values were obtained by sample treatment with neutral detergent and acid solutions for 1 h, at 100 °C, respectively, and subsequent determination of the nitrogen content in each residue [14].

2.4. Statistical Analysis

A completely randomised block design was used, with four treatments and eight replicates; the blocks were formed with regard to the initial body weight of the animals.

The results were subjected to analysis of variance (ANOVA) by the Proc GLM and regression by Proc Reg, at 5% significance level, using SAS software (2001). Homogeneity of variance between treatments was assumed, and the degrees of freedom were estimated using the Kenward-Roger method. The choice of the best model was performed based on the coefficient of determination adopting the following statistical model:

$$Y_{ijk} = \mu + T_{Ai} + A_{j} + B_{k} + e_{ijk},$$

where:

$$Y_{ijk} = \text{observation referring the block k, animal j in the content of soybean meal substitution by cottonseed cake;}$$

$$\mu = \text{overall average;}$$

$$T_{Ai} = \text{effect of the content of soybean meal substitution by cottonseed cake i (i = 1, 2, 3 and 4);}$$

$$A_{j} = \text{effect of the animal j (j = 1, 2, 3, 4, 5, 6, 7, 8..., 32);}$$

$$B_{k} = \text{effect of the block k (k = 1, 2, 3, 4, 5, 6, 7 and 8);}$$

$$e_{ijk} = \text{random error at each observation.}$$

3. Results

3.1. Nutrient Intake

The average intakes of water (H₂O, L/day), dry matter (DMI), ether extract (EEI), neutral detergent fiber (NDFI) and total digestible nutrients (TDNI) were not modified by cottonseed cake levels in rams diets ($p > 0.05$). The inclusion of cottonseed cake in the diet caused linear decreases in CPI ($p < 0.001$) and NFCI ($p = 0.005$), and DMI, CPI, NFCI expressed in g/kg BW and g/kg BW⁰.⁷⁵ ($p < 0.01$) (Table 3).
Table 3. Average intakes of dry matter (DMI), crude protein (CPI), ether extract (EEI), neutral detergent fiber (NDFI), non-fibrous carbohydrates (NFCI), total carbohydrates (TCI), total digestible nutrients (TDNI), indigestible neutral detergent fiber (iNDFI) and water (H₂O) by rams fed diets containing different levels of cottonseed cake.

| Variable | Cottonseed Cake (g/kg DM) | SEM | Regression Equation | p-Value |
|----------|--------------------------|-----|---------------------|---------|
|          | 0 | 70 | 140 | 210 | L | Q |
| H₂O      | 4071 | 3642 | 3388 | 2975 | 237.8 | Y = 3519 | 0.114 | 0.986 |
| DMI      | 1113 | 1247 | 1088 | 950.8 | 55.38 | Y = 1100 | 0.205 | 0.235 |
| CPI      | 92.13 | 77.36 | 79.46 | 53.79 | 3.858 | Y = 92.623 – 0.1613x | <0.001 | 0.400 |
| EEI      | 37.68 | 40.61 | 42.55 | 41.35 | 1.494 | Y = 40.55 | 0.362 | 0.513 |
| NDFI     | 353.0 | 370.9 | 389.1 | 305.1 | 16.50 | Y = 354.5 | 0.405 | 0.136 |
| NFCI     | 413.3 | 391.4 | 370.0 | 301.3 | 14.39 | Y = 422.61 – 0.5106x | 0.005 | 0.382 |
| TCI      | 766.3 | 762.4 | 759.2 | 606.4 | 29.93 | Y = 723.6 | 0.076 | 0.215 |
| TDNI     | 610.7 | 599.0 | 567.7 | 484.3 | 24.13 | Y = 565.4 | 0.067 | 0.462 |
| DMI      | 31.16 | 30.38 | 30.80 | 24.96 | 0.964 | Y = 32.052 – 0.026x | 0.034 | 0.176 |
| CPI      | 2.58 | 2.24 | 2.30 | 1.56 | 0.101 | Y = 2.62 – 0.0043x | <0.001 | 0.239 |
| EEI      | 1.05 | 1.15 | 1.23 | 1.22 | 0.032 | Y = 1.16 | 0.054 | 0.381 |
| NDFI     | 9.94 | 10.65 | 11.27 | 8.89 | 0.416 | Y = 10.19 | 0.498 | 0.072 |
| NFCI     | 11.56 | 11.18 | 10.73 | 8.87 | 0.340 | Y = 11.863 – 0.0122x | 0.004 | 0.239 |
| TCI      | 21.50 | 21.83 | 22.00 | 17.76 | 0.731 | Y = 20.77 | 0.092 | 0.118 |
| TDNI     | 17.11 | 17.10 | 16.48 | 14.14 | 0.579 | Y = 16.21 | 0.740 | 0.863 |
| DMI      | 427.6 | 414.0 | 418.1 | 337.9 | 12.71 | Y = 439.15 – 0.3786x | 0.018 | 0.169 |
| CPI      | 35.40 | 30.41 | 31.24 | 21.21 | 1.360 | Y = 35.826 – 0.0596x | <0.001 | 0.250 |
| EEI      | 14.46 | 15.77 | 16.72 | 16.48 | 0.421 | Y = 15.86 | 0.072 | 0.364 |
| NDFI     | 136.2 | 145.1 | 153.0 | 120.6 | 5.56 | Y = 138.7 | 0.433 | 0.070 |
| NFCI     | 158.7 | 152.5 | 145.6 | 120.0 | 4.49 | Y = 162.65 – 0.1757x | 0.002 | 0.225 |
| TCI      | 228.4 | 210.1 | 222.2 | 177.6 | 8.90 | Y = 209.6 | 0.084 | 0.460 |
| TDNI     | 234.8 | 233.2 | 223.7 | 191.7 | 7.73 | Y = 220.8 | 0.075 | 0.320 |

3.2. Nutrient Digestibility

The apparent digestibility data of DM, CP, EED, NDF, NFC and TC of experimental diets are shown in Table 4. It can be observed that the apparent digestibility of DM, CP and NFC decreased linearly (p < 0.01).

Table 4. Apparent digestibility of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), non-fibrous carbohydrates (NFC) and total carbohydrate (TC) by rams fed diets containing different levels of cottonseed cake.

| Variable | Cottonseed Cake (g/kg DM) | SEM | Regression Equation | p-Value |
|----------|--------------------------|-----|---------------------|---------|
|          | 0 | 70 | 140 | 210 | L | Q |
| DM       | 656.6 | 649.2 | 629.2 | 593.7 | 8.22 | Ŷ = 663.48 – 0.2981x | <0.001 | 0.052 |
| CP       | 705.6 | 700.4 | 662.9 | 615.2 | 9.86 | Ŷ = 717.33 – 0.4410x | <0.001 | 0.081 |
| EE       | 779.2 | 809.5 | 775.1 | 774.6 | 5.52 | Ŷ = 784.6 | 0.319 | 0.152 |
| NDF      | 498.9 | 543.1 | 514.7 | 508.7 | 12.92 | Ŷ = 516.4 | 0.993 | 0.358 |
| NFC      | 721.3 | 714.5 | 705.9 | 660.9 | 5.15 | Ŷ = 729.12 – 0.2711x | 0.002 | 0.131 |
| TC       | 622.6 | 632.8 | 590.2 | 583.1 | 7.71 | Ŷ = 607.2 | 0.079 | 0.825 |
3.3. Nutrient Fractions

The carbohydrate fractions (\(A + B_1\)), (\(B_2\)) and C of diets containing cottonseed cake are shown in Table 5. Regarding carbohydrate fractionation, it was observed that the inclusion of cottonseed cake caused a linear reduction (\(p = 0.006\)) for the (\(A + B_1\)) fraction, but did not present a significant effect (\(p = 0.250\)) on the \(B_2\) fraction, with an average of 142.5 (Table 5).

Table 5. Average values of total carbohydrate (TC), fractions (\(A + B_1\)), (\(B_2\)) and C intakes of diets to rams containing different levels of cottonseed cake.

| Variable | Cottonseed Cake (g/kg DM) | SEM | Regression Equation | p-Value |
|----------|---------------------------|-----|---------------------|---------|
|          | 0 | 70 | 140 | 210 |
| TC       | 766.3 | 762.4 | 759.2 | 606.4 | 29.93 | \(\hat{Y} = 723.6\) | 0.076 | 0.215 |
| Fraction A + B₁ | 533.8 | 513.0 | 479.8 | 377.8 | 20.92 | \(\hat{Y} = 551.28 - 0.716x\) | 0.006 | 0.292 |
| Fraction B₂ | 143.7 | 147.2 | 151.7 | 125.5 | 5.88 | \(\hat{Y} = 142.5\) | 0.297 | 0.250 |
| Fraction C | 86.8 | 102.2 | 127.7 | 103.1 | 5.12 | \(\hat{Y} = 83.79 + 0.5349x - 0.002x^2\) | 0.083 | 0.039 |

\(^{1}\) g of DM.

The inclusion of cottonseed cake caused a quadratic effect on carbohydrate fraction C (\(p = 0.039\)) with maximum amount of fraction C of 129.55 g in 133.73 g/kg of cottonseed cake inclusion (Table 5).

There was a significant effect (\(p < 0.001\)) on nitrogen fractions A, B3 and C with cottonseed cake inclusion in the diets (Table 6). The fractions A and B3 decreased linearly (\(p < 0.001\)), while fraction C increased linearly (\(p < 0.001\)) when soybean meal was replaced with cottonseed cake. The cottonseed cake inclusion did not affect (\(p = 0.194\)) the intake of nitrogen fractions B₁ + B₂ (Table 6).

Table 6. Average values of the nitrogen fractions (A, \(B_1 + B_2\), B₃ and C) of diets to rams containing different levels of cottonseed cake.

| Variable | Cottonseed Cake (g/kg DM) | SEM | Regression Equation | p-Value |
|----------|---------------------------|-----|---------------------|---------|
|          | 0 | 70 | 140 | 210 |
| Fraction A₁ | 46.14 | 38.68 | 33.73 | 17.63 | 2.298 | \(\hat{Y} = 47.917 - 0.1293x\) | <0.001 | 0.074 |
| Fraction B₁ + B₂₁ | 11.16 | 10.08 | 13.00 | 9.26 | 0.537 | \(\hat{Y} = 10.8\) | 0.541 | 0.194 |
| Fraction B₃₁ | 27.12 | 19.21 | 19.68 | 12.25 | 1.204 | \(\hat{Y} = 26.186 - 0.0631x\) | <0.001 | 0.876 |
| Fraction C₁ | 7.86 | 9.45 | 13.01 | 14.64 | 1.097 | \(\hat{Y} = 7.355 + 0.0341x\) | <0.001 | 0.043 |

\(^{1}\) g of DM.

4. Discussion

The NDF content in the cottonseed cake provided increases of 25.6 g/kg in NDF and 17.8 g/kg in iNDF and decreases of 22.3 g/kg in NFC and 24.1 g/kg in TDNc in the treatment diets with its inclusion. This resulted in decreases in the DM intake in g/kg BW and g/kg BW\(^{0.75}\), and consequently in the intakes of CP and NFC in g/day, g/kg BW and g/kg BW\(^{0.75}\).

Several authors have reported that the increase in the iNDF content in diets provided a longer retention time in the rumen, which probably could have occurred with cottonseed cake inclusion, this prompted to increase cholecystokinin (CCK) secretion in the blood plasma, contributed to the satiety of the animal, inhibiting the gastrointestinal tract motility and resulting in a lower digestion rate [25–29]. This prevented gastrointestinal tract emptying, which distended the rumen-reticulum and reduced voluntary consumption by filling [25–27]. On the other hand, the faster the ruminal degradation process of the \(B_2\) fraction (i.e., the potentially digestible fiber, the faster the indigested ruminal residue (iNDF) leaves the rumen), which releases space for increased feed intake by the animal. In this study, there was no effect on the fraction \(B_2\) with cottonseed cake inclusion. However, its addition caused an increase in the intake of fraction C, which may have reduced the intake of DM proportional to body weight and decreased the apparent digestibility of DM, which may negatively impact animal performance [28].
The decreasing trend in TDN intake in g/day ($p = 0.067$) is possibly related to the decreased intake of digestible fractions that compose the TDN equation. However, the mean TDN intake was 565.4 g/day, lower than that recommended [13], which is 680 g TDN/day; none of the treatments reached the required TDN value.

Inclusion of 210 g of cottonseed cake caused a decrease in water intake of 26.92% compared to the diet with no inclusion, but without significant effect ($p = 0.114$). There is a relationship between H2OI and MSI. In ovine, the normal water intake is two to three times greater than MSI [13,29], in this research was observed that water intake/MSI had ranging from 2.92 to 3.65 times, the highest value was observed without inclusion and the lowest value in the treatment 70 g/kg of cottonseed cake inclusion.

The CP digestibility reduced with cottonseed inclusion in the diets, probably due to the higher ADIN (fraction C), which is bound to the fibers and cannot be degraded by microorganisms or absorbed by the small intestine, which results in losses of N in feces. Similar data were found by [30] when evaluating the physicochemical characteristics of protein sources, such as soybean meal, peanut meal, canola meal and cottonseed meal.

The increase in the EE concentration provided by the inclusion of cottonseed cake can increase the digestibility of this fraction and decrease the digestibility of NDF. However, it is important to emphasise that EE content did not affect NDF digestibility, probably due to the available form of the EE in cottonseed cake, that had no negative effects on the development and activity of the microbial population in the rumen, and hence, on the degradability of NDF [31].

The DM digestibility decreased, probably due to a reduction in NFCI and CPI, associated with the increase in iNDF content in the diets, which reduced the passage rate in the rumen, increasing the retention time and favouring fiber degradation by allowing longer degradation by ruminal microorganisms [32].

Therefore, it is possible that the NDF content of cottonseed cake may have contributed to the reduced, digestibility coefficients, due to the non-associative effects between nutrients and the physical conditions of the ingested particles.

Considering the fractionation of carbohydrates, it was observed that each gram of cottonseed cake included in the diet reduced the $A + B_1$ fraction by 0.358 g; this is probably associated with the decrease in CP and NFC contents and the increase in NDF content in cottonseed cake. The TC, as well as FC, represented by hemicellulose and cellulose together with lignin, are essential for ruminants because they provide energy for the ruminal microorganisms to produce short-chain fatty acids, which is the energy source used by the animal [24,25,33].

The fraction $A + B_1$ that represents the NFC contents (i.e., soluble carbohydrates with degradation rate greater than the passage rate) was reduced by 13.11% with cottonseed cake inclusion. This result is not interesting because this fraction is the main source of readily available energy in the rumen to stimulate the growth of ruminal microorganisms [34]. According to nitrogen availability, these carbohydrates affect the metabolism and microbial multiplication and, consequently, feed digestion, microbial protein production and the number of amino acids and peptides available for absorption in the small intestine [27].

The carbohydrate fraction $B_2$, with a slow rate of degradation, together with fraction C (indigestible), generally affect the animals ‘ingestion by rumen filling, which can reduce the animals’ performance [25]. In this study, the fraction $B_2$ and fraction C were more available with cottonseed cake inclusion, however the inclusion showed no effect in the animal’s intake of the fraction $B_2$ indicating that the inclusion of the cottonseed by-product did not increase the energy from the fiber for the animal. However, a significant increase in fraction C was observed, this fraction is not an energy source, but it acts directly on the filling of the gastrointestinal tract and modified the passage rate affecting the intake as previously reported.

The determination of protein fractions in the feed allows estimation of their respective contents, besides the greater or lesser escape of ruminal nitrogen. Based on this information, it is possible to develop nutritional strategies to improve the use of nitrogen by the ruminal and animal microorganisms [35].
Therefore, it has been observed that the inclusion of cottonseed cake decreased in fraction A, which represents the NPN. It is not interesting because it is the protein fraction readily available in the rumen, promoting an asynchrony between the availability of nitrogen and carbonic skeleton for ruminal microorganisms (for their growth and development), which can causing excess nitrogen in the rumen that tends to be absorbed and transformed into urea that probably will be excreted [35].

The intake of fraction \((B_1 + B_2)\), corresponding to the soluble and insoluble proteins, was not affected by cottonseed inclusion, an interesting result because it is a fraction that is extensively degraded in the rumen and contributes to the nitrogen requirements of the ruminal microorganisms. Rapid proteolysis in the rumen may accumulate peptides that may reduce the degradation of other proteins by negative feedback and/or may allow their escape to the small intestine for absorption by the animal [20,27].

Proteins bound to the cell wall with slow ruminal degradation, represented by fraction \(B_3\), decreased with cottonseed inclusion. Although this fraction is digestible, it has a ruminal degradation rate of only 0.02 to 1.0%/h [20,36], which results in the higher ADIN content (fraction C), observed in this study.

The formulation of total mixed ration when considering only the nitrogen total requirements of ruminal microorganisms with CP levels can be misleading when they do not consider the fractions of carbohydrates and proteins are responsible for the leakage of rumen nitrogen and can result in incorrect predictions of animal performance [36,37]. The knowledge of fractions must be considered to provide the greatest efficiency in the use of the diets formulated.

5. Conclusions

The inclusion of cottonseed cake in the rams feed should be carried out with caution because this by-product promotes linear reduction in intake and digestibility nutrient, in addition to modifying the amounts of digestible and indigestible fractions in the diets.

**Author Contributions:** Conceptualisation, F.N., A.Z., M.R., D.F., A.S., M.P., H.P., L.G. and T.L.; methodology, F.N., M.R. and R.R.; software, M.P., H.P., A.L. and T.L.; validation, A.Z., M.R., D.F., A.S., M.P., A.L. and R.R.; formal analysis, A.Z., F.N., D.F. and A.L.; investigation, F.N., M.R., M.P., H.P., L.G., R.R. and T.L.; resources, F.N., A.S., M.P., H.P. and R.R.; data curation, A.Z., M.R., D.F., A.S., M.P. and T.L.; writing—original draft preparation, F.N., A.Z., M.R., D.F., A.S., M.P., H.P., A.L. and R.R.; data curation, F.N., A.Z., M.R., D.F., A.S., M.P., H.P., L.G., R.R. and T.L. writing—review and editing, F.N., A.Z., D.F., A.S., M.P., H.P., A.L. and R.R.; visualisation, F.N., A.Z., M.R., D.F., A.S., M.P., H.P., L.G., A.L., R.R. and T.L.; supervision, M.R., A.Z., D.F., A.S., M.P. and R.R.; project administration, M.R., A.Z., M.P. and R.R.; funding acquisition, M.R. and A.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received funding from the Foundation for Research Scientific and Technological Development of Maranhão (Fundaçao de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão—FAPEMA) and the Federal Institute of Education, Science and Technology of Rondônia (Instituto Federal de Educação, Ciência e Tecnologia de Rondônia—IFRO; Departamento de Pesquisa—DEPESP/Colorado do Oeste).

**Acknowledgments:** This research was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES-Brazil), the Maranhão State Research Foundation (FAPEMA-Brazil), and the Federal Institute of Education, Science and Technology of Rondônia (Instituto Federal de Educação, Ciência e Tecnologia de Rondônia—IFRO; Departamento de Pesquisa—DEPESP/Colorado do Oeste).

**Conflicts of Interest:** The authors declare no conflict of interest.

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