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The Effect of Cotton Lint from Agribusiness in Diets on Intake, Digestibility, Nitrogen Balance, Blood Metabolites and Ingestive Behaviour of Rams

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Simple Summary: The confinement system using traditional ingredients to feed animals, such as ground corn and soybean meal, has a high cost. Thus, alternative feeds that are cheaper, available in the region, and that present good nutritional quality are recommended. In this way, the residue, cottonseed lint, which is an agroindustry by-product, is an alternative feed that is cheaper than traditional feed ingredients. However, it has a high content of fiber and lignin, which is unattractive for non-ruminants' diets, but may be an interesting option in ruminant feeding.

Abstract: This study aimed to evaluate intake, digestibility, nitrogen balance, ingestive behavior, and blood parameters of sheep fed with cotton lint levels. Twenty rams weighing 30.2 ± 3.7 kg and aged 12 ± 1.3 months were distributed in a completely randomized design, with four treatments and five repetitions. Diets consisted of 50% roughage and 50% concentrate. The treatments consisted of replacing corn with cotton lint at levels of 0, 70, 140, and 210 g/kg of dry matter (DM) of the diets. The animals' feeding behavior was determined in the last three days of the experimental period. Data were subjected to regression analysis. Decreased linear effect ($p < 0.05$) was observed for the nutritional fraction's intake. However, neutral detergent fibre (NDF) intake and plasma urea-N were not affected ($p > 0.05$) by lint levels. Apparent digestibility of DM, crude protein (CP), ethereal extract (EE), and non-fibrous carbohydrate (NFC) were affected ($p < 0.05$), except for total carbohydrate. There was a decreased linear effect ($p < 0.05$) for the intake efficiency of DM and NDF in g/h. The nitrogen balance (g/day) and glucose levels (mg/dL) were reduced with the addition of lint in the diet. The addition of cotton lint up to 70 g/kg in DM can be used over a short-term period.

Keywords: alternative feed; degradability; fractions; sustainability

1. Introduction

The world population is growing, and thus there is a greater demand for food production. Thus, the productive chain is searching for alternatives to optimize production. In the food sector, the meat industry seeks to optimize its production using the feedlot, a technique for finishing animals faster that requires high technology. However, one of the primary issues is to limit the feed cost, which is about 70–80% of the final cost [1].

In view of the above, alternative products that replace traditional feeds, such as corn and soybean meal, are often a solution to reduce costs [1], and in this case, the residues from the cotton agribusiness have gained prominence in recent years [2]. According to Moreira et al. [3], the cotton culture can originate several products, from the direct ones, such as lint and oil, to the by-products, such as cottonseed, hull, and cottonseed meal.

The residual cottonseed lint is considered one of the by-products of cotton, obtained through the delinting process, where the fiber is separated from the cottonseed through chemical or physical treatments. This residue of lignocellulosic fibers is generally used in the manufacture of paper money, and the production of cotton wool and surgical fabrics. The lint content varies according to the cotton cultivar and the extraction process, ranging from 4 to 8% dry matter of the cottonseed after extraction [4]. The cottonseed lint residue has a high content of metabolizable energy, 13.29 kJ/kg DM, due to the high level of ether extract 268.7 g/kg DM [5].

According to the Companhia Nacional de Abastecimento (CONAB) [6], the 2017 harvest in the state of Mato Grosso, Brazil accounted for 56.5% of Brazilian cotton production, being the largest cotton producer in Brazil, with its by-products demonstrating a high production scale. Every year an average of 35 million hectares of cotton are planted across the planet and world demand has gradually increased since the 1950s, at an average annual growth of 2% [6]. The trend of production growth this year should consolidate Brazil as the fourth-largest producer and second-largest exporter. In the harvest planted in 2019, 3 million tons were produced. In 2017, 1.5 million tons were produced. For 2022, production is estimated between 2.6 and 2.8 million tons. There are many studies developed with cottonseed, hull and meal, whereas they are scarce or non-existent in the literature concerning lint for animal feed [3,7,8].

Thus, knowledge of the physical and chemical properties of this by-product is extremely important since it has specific characteristics in relation to other cotton by-products. Therefore, studies assessing both the chemical composition of by-products and animal tests help in the evaluation of feed, since it allows for determining the nutritional value of alternative feeds [9–11].

In view of the above, we hypothesised that by-products such as cottonseed lint can replace conventional ingredients in the diet to some level, and due to the chemical composition, lint can be an alternative ingredient to ground corn.

In this context, the aim of this study was to evaluate the addition of cotton lint in the diet of confined sheep on intake, feeding behavior, digestibility, nitrogen balance, and blood parameters.

2. Materials and Methods

2.1. Study Location

This study was conducted in the metabolism shed of the experimental area of the Animal Science Faculty at the Federal University of Mato Grosso, Rondonópolis Campus, Mato Grosso State, Brazil (16°28′ S, 50°34′ W), under the Federal University of Mato Grosso Animal Use and Care Committee (Protocol Number 23108.046399/13-4).

2.2. Animals, General Procedures, and Experimental Diets

A total of 20 mixed-breed rams with an average age of 12 $\pm$ 1.3 months and average weight of 30.2 $\pm$ 3.7 kg were used in a completely randomized design with four treatments and five replicates. The experiment was performed in April 2015 and lasted 21 days, with 15 days for adaptation to diets, environment, and management, and 6 days for sample collection. The experimental diets consisted of 50% corn silage and 50% concentrate on a DM basis, formulated to meet the nutritional needs of animals weighing 30 kg and gaining 150 g/day [12]. Soybean meal, ground corn, lint, and urea were used in the formulation of concentrates. The lint was derived from a cotton processing company located in the city of Primavera do Leste, MT, Brazil. The treatments consisted of replacing the ground corn with cottonseed lint at levels of 0, 70, 140, and 210 g/kg dry matter of the diets (Table 1).

Table 1. Chemical composition of experimental diets ingredients, maize silage (MS), ground corn (GC), soybean meal (SM), cottonseed lint (LIN), and urea.

Chemical Composition of Ingredients (g/kg DM)					
	MS	**GC**	**SM**	**LIN**	**Urea**
Dry matter [1]	327.30	910.00	896.70	900.20	990
Crude protein	58.90	81.30	480.10	29.70	2812.5
Ethereal extract	31.60	53.60	130.05	18.90	0
Ash	59.30	11.50	67.60	67.80	0
NDFap [2]	454.44	112.87	105.41	407.85	0
NDF [3]	541.80	125.25	128.74	487.80	0
ADF [4]	324.50	31.33	83.61	280.50	0
NFC [5]	395.80	740.08	216.4	475.80	0
TC [6]	850.20	853.36	322.24	883.60	0

[1] Dry matter in g/kg Natural matter; [2] NDFap: Neutral detergent fibre corrected to ash and protein; [3] NDF: Neutral detergent fibre; [4] ADF: Acid detergent fiber; [5] NFC: Non-fibrous carbohydrate; [6] TC: Total carbohydrate.

The NDF corrections for ash and protein (NDFap) were performed according to Licitra et al. [13]. The total carbohydrates (TC) were calculated according to Sniffen et al. [14] as portrayed in Equation (1):

$$\text{TC} = 100 - (\%\text{CP} + \%\text{Ash} + \%\text{EE}) \tag{1}$$

The non-fibrous carbohydrates (NFC) were calculated in the manner proposed to Hall et al. [15] according to Equation (2):

$$\text{NFC} = 100 - [\%\text{CP} + \%\text{NDFap} + \%\text{EE} + \%\text{ash}]. \tag{2}$$

Degradability data for the fractions of the ingredients were also estimated. The protein fractions (A, B1 + B2, B3, and C) were determined according to Licitra et al. [13]; carbohydrate fractions (A + B1, B2, and C) were proposed by Hall et al. [15] and Cabral et al. [16]; and estimation of the degradation of DM, CP, and NDF (Table 2) was according to Orskov and McDonald [17].

Table 2. Carbohydrate and protein fractionation, and degradation parameters of DM, CP, NDF from cottonseed lint.

	MS *	**GC ***	**SM**	**LIN**
Carbohydrate Fractionation (%TC)				
$A + B_1$	34.00	76.60	73.58	54.75
B_2	45.39	21.50	17.32	15.53
C	20.61	1.90	9.10	29.72
Protein Fractionation (% CP)				
A	32.71	11.70	18.72	33.83
$B_1 + B_2$	44.54	75.12	75.47	37.58
B_3	10.41	11.13	3.21	5.79
C	12.34	2.05	2.60	22.80
DM Degradation Parameters				
a	28.60	19.60	13.29	10.21
b	50.44	74.08	92.04	59.91
kd	0.036	0.072	0.003	0.081
CP Degradation Parameters				
a	56.34	23.27	7.65	4.03
b	33.11	55.37	90.74	74.20
kd	0.040	0.054	0.070	0.187

Table 2. *Cont.*

	MS *	GC *	SM	LIN
NDF Degradation Parameters				
a	0.89	3.93	10.33	5.65
b	67.25	39.61	52.24	55.99
kd	0.051	0.243	0.098	0.112

* CQBAL datas [18]; TC = Total carbohydrate; A + B_1 = Non-fibrous carbohydrate (%TC); B_2 = potentially digestible neutral detergent fibre (%TC); C = unavailable; A = non-protein nitrogen; B_1 + B_2 = true protein degradable in the rumen; B_2 = true protein with intermediate degradation rate in the rumen; B_3 = true protein with slow degradation rate in the rumen, C = unavailable or cell-wall bound true protein; (a) water-soluble fraction; (b) water-insoluble fraction potentially degradable in the rumen as a function of time; (kd) rate of degradation per fermentative action of b.

The rams were dewormed with Invermectin 1%-Ivomec-Merial® and were placed in metabolic cages provided with feeders, feed troughs, and drinking fountains. The animals were offered *ad libitum* water and mineral salt (Coperphós ovino®) with guaranteed levels (g/kg) as follows: zinc 30.00; calcium 17.70; manganese 12.00; phosphorus 8.00; fluorine (maximum) 8.00; iodine 6.00; copper 5.50; sulphur 2.00; sodium 0.40; selenium 0.15.

In the last three days, the ingestive behavior and the water intake of each animal were quantified by the daily difference between the supplied volume and the leftover water in graduated buckets. The buckets were washed at every use and two of them were allocated in the installation with water in order to measure evaporation losses [19].

Feed intake was measured daily by the difference in weight between the offered feed and the leftovers. The amounts of daily diets supplied per animal were adjusted in order to allow 10% of leftovers on a natural matter basis.

In the last six days of the experiment, 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. The diets were fed twice a day, with an interval of 8 hours between them, in a total mixed ratio (t 7.30 a.m. and 3.30 p.m.) (Table 3).

Table 3. Proportion of ingredients and chemical composition of diets with cottonseed lint levels, and average of body weight animals fed each level.

Ingredients (g/kg DM)	Cottonseed Lint Levels (g/kg)			
	0	**70**	**140**	**210**
Maize silage	500.00	500.00	500.00	500.00
Ground corn	424.00	352.20	280.70	209.00
Soybean meal	76.00	76.30	76.20	76.30
Lint	0.00	70.00	140.00	210.00
Urea	0.00	1.50	3.10	4.70
Chemical composition of diets (g/kg DM)				
Dry matter [1]	617.64	617.07	616.51	615.95
Organic matter	960.05	958.87	957.90	956.93
Crude protein	100.41	101.01	101.73	102.53
Ethereal extract	48.41	45.92	43.40	40.89
Ash	39.95	41.13	42.10	43.07
NDFap [2]	283.09	303.57	324.03	344.50
NDF [3]	333.79	358.98	384.16	409.34
ADF [4]	181.89	199.30	216.69	234.08
Hemicelullose	151.90	159.68	167.47	175.26
NFC [5]	528.14	508.37	488.74	469.01
TC [6]	811.41	812.09	812.90	813.60
TDN [7] estimated	747.31	733.75	720.20	706.65
Metabolisable energy [8] (Mj/kgDM)	11.25	11.05	10.84	10.63

Table 3. *Cont.*

Ingredients (g/kg DM)	Cottonseed Lint Levels (g/kg)			
	0	**70**	**140**	**210**
Animal's body weight in each group				
Body weight (kg)	29.19	28.91	32.63	29.90

[1] Dry matter in g/kg Natural matter; [2] NDFap: Neutral detergent fiber corrected to ash and protein; [3] NDF: Neutral detergent fibre; [4] ADF: Acid detergent fibre; [5] NFC: Non-fibrous carbohydrate; [6] TC: Total carbohydrate. [7] TDN: Total digestible nutrients. [8] Metabolizable energy was estimated using the equation provided by the NRC [12].

The total collection of feces was performed with the aid of individual collector bags adapted to the animals through a breastplate. The samples were collected twice a day, always before the provided diet. The samples were then weighed and stored under −20 °C for further analyses.

2.3. Chemical, Intake, Digestibility, Nitrogen Balance and Blood Analyses

The samples of silage, lint, concentrate, leftovers, and feces were stored in polyethylene containers for further analysis of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), mineral matter (MM), neutral detergent fiber (NDF) and acid detergent fiber (ADF), according to methodologies described by Silva and Queiroz [20].

The DM and nutrient digestibility coefficients of the diets were calculated based on the difference between the amounts ingested and excreted in the feces.

The digestibility coefficients (DC) from DM, CP, NDF, and EE were calculated through the Equation (3):

$$\mathrm{DC} = \frac{(\text{kg of the portion ingested} - \text{kg of the portion excreted})}{\text{kg of the portion ingested}} \times 100 \quad (3)$$

Samples of diets, feces and leftovers were stored frozen. Before performing the analysis, they were thawed, mixed, homogenized, and pre-dried in a convection oven at 65 °C, then ground in a Thomas Wiley mill on 1 mm sieves.

The urine of each animal was collected in the last three days and put in buckets arranged under the cages containing 50 mL of 50% hydrochloric acid in order to avoid losses by volatilization of urinary NH_3. The total urine volume of each animal was recorded and a 10% aliquot was taken, which was packed in plastic flasks and frozen for further analysis. Nitrogen balance (NB) was obtained by the difference between total nitrogen ingested and total nitrogen excreted in feces and in urine [21].

Plasmatic ureic nitrogen (PUN) was determined through blood collection, in Vacutainer tubes with ethylenediamine tetra acetic acid (EDTA), which was performed 3 h after feeding in the last day of collection, obtaining the plasma, which was analyzed by colorimetric-enzymatic method on a biochemical analyzer (Thermo Plate TP Analyser®) for urea determination, using commercial kits (Urea 500, Labtest®).

For reading the glucose concentration, a digital glucose meter (Accu-Chek®) equipped with test tape (tape reagent) was used. The measurement was performed immediately after collecting blood from the animals.

2.4. Assessment of Feeding Behaviour

The animals' feeding behavior was determined in the last three days of the experimental period by quantifying the time intervals spent on feeding, rumination, and leisure throughout 24 h [22]. To record the time of these activities, visual observation of animals was adopted every 10 min.

Trained observers were strategically positioned in the shed so as not to disturb the animals. In the same period, the number of cud chewings (MMnb, n/bolus) and the time taken to ruminate each bolus (MMtb, sec/bolus) were counted using a digital stopwatch. The minimum and maximum temperature averages were obtained every 1 h, within 24 h.

Chewing averages and rumination time were obtained through observations of rumen boluses made every 30 min, within each 24-h evaluation period [23]. The time and number of chews for each rumen bolus per animal within this period were recorded. The variables g DM/bolus and NDF/bolus were obtained by dividing the average individual intake of each fraction by the number of boluses ruminated per day (within 24 h).

The efficiencies of feeding and rumination, in g of DM/h and g of NDF/h were obtained by dividing the average daily intake of DM and NDF divided by the time spent in feeding and rumination within 24 h, respectively. These and other variables, such as the number of boluses ruminated per day (NBR), the total chewing time (TCT), and the number of cud chewings per day (CCD) were obtained according to the methodology described by Polli et al. [22] and Burguer et al. [23]. During the night data collection, the environment was maintained with artificial lighting.

The number of eating, rumination, and idleness periods were counted by the number of activity sequences observed in the worksheet. The average daily duration of these activity periods was calculated by dividing the total duration of each activity (eating, rumination, and idleness in min/day) by their respective number of periods.

2.5. Statistical Analysis

The experiment was performed in a completely randomized design with four treatments and five replicates (animals) per treatment. Data on intake, apparent digestibility, nitrogen balance, blood glucose and serum urea were submitted to analysis of variance and regression testing. The statistical model is represented by Equation (4):

$$\mathrm{Yij} = \mu + \mathrm{Ti} + \beta\left(\mathrm{Xi} - \overline{\mathrm{X}}\right) + \mathrm{eij} \tag{4}$$

where Yij was the observed dependent variable, μ—overall average; Ti—fixed effect of treatment i; in which i = 0, 70, 140 and 210 g/kg; β—regression coefficient or functional relationship with the covariate; Xi—observed value of the covariate (body weight) applied to the experimental unit; $\overline{\mathrm{X}}$—average of the covariate; j—random error associated with each observation and j repetitions, where j = 1, 2, 3, 4, and 5.

The data were subjected to analysis of variance using the PROC GLM command of the SAS statistical package (SAS version 9.1, 2003), and the means were subjected to regression analysis using the PROC REG command of the SAS® (9.1) statistical package (SAS University Edition). Analysis of ingestive behavior also included a *t*-test in SAS® (9.1) to verify the effect of the period (daytime and nighttime). Significance was declared when $p \leq 0.05$.

3. Results

3.1. Nutrient Intake

The addition of cotton lint promoted a decreasing linear effect ($p < 0.01$) in the intake of CP and NFC (Table 4). Considering the diet effectively consumed, despite the diets being completely supplied, it can be observed that there was selectivity of the diet by the animals (Table 4), indicating that the addition of lint at the 210 g/kg level led to the almost exclusive intake of roughage. The diets effectively consumed were modified with the addition of lint and caused a linear decreasing effect ($p < 0.01$) in the fractions CP, EE, NFC, but the lint promoted a linear increasing effect ($p < 0.01$) in the NDF and TC of (Table 4).

3.2. Nutrient Digestibility

The apparent digestibility of DM, CP, EE, and NFC presented a decreased linear effect ($p < 0.05$) with the addition of the lint in the diet (Table 5). In contrast, the NDF digestibility presented an increasing linear effect ($p < 0.0001$) and the TC digestibility was not influenced ($p = 0.5036$) by the addition of lint.

Table 4. 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 (H_2OI) of rams fed with cottonseed lint inclusion levels diets.

Variable	Cottonseed Lint Levels (g/kg)				SEM [1]	Regression Equation	p-Value	
	0	70	140	210			L [2]	Q [3]
Intake g/day								
DMI	986.56	796.68	722.31	517.81	23.78	$\hat{Y}$ = 755.84	0.114	0.986
CPI	91.58	72.59	55.61	39.10	3.858	$\hat{Y}$ = 92.623 − 0.1613x	<0.001	0.400
NDFI	210.90	195.87	217.94	183.17	1.494	$\hat{Y}$ =201.97	0.362	0.513
EEI	57.38	44.01	37.87	26.37	16.50	$\hat{Y}$ = 41.41	0.405	0.136
NFCI	590.04	454.01	383.37	253.21	14.39	$\hat{Y}$ = 422.61 − 0.5106x	0.005	0.382
TCI	801.20	650.05	601.45	436.47	29.93	$\hat{Y}$ = 622.29	0.076	0.215
H_2OI	641.88	838.25	468.75	638.33	24.13	$\hat{Y}$ = 646.80	0.067	0.462
Relation between intake and body weight g/kg								
DMI BW	24.67	24.30	20.71	17.77	0.964	$\hat{Y}$ = 32.052 − 0.026x	0.034	0.176
DMI $BW^{0,75}$	77.84	64.42	53.24	41.92	0.101	$\hat{Y}$ = 2.62 − 0.0043x	<0.001	0.239
NDFI BW	7.10	6.89	6.74	6.43	0.032	$\hat{Y}$ = 6.79	0.054	0.381
H_2OI BW	22.24	29.13	15.93	23.69	0.416	$\hat{Y}$ = 22.75	0.498	0.072
Diet effectively consumed g/kg								
CPI	92.17	90.89	76.50	68.39	2.438	Y = 94.84 − 1.225x	<0.001	0.081
NDFI	205.24	244.14	301.22	353.93	13.49	Y = 200.66 + 7.189x	<0.001	0.414
EEI	59.36	55.52	52.64	51.16	0.934	Y = 58.795 − 0.393x	0.003	0.389
NFCI	606.70	571.70	531.70	489.44	10.80	Y = 608.65 − 5.597x	<0.001	0.678
TCI	812.20	816.06	833.13	843.55	3.011	Y = 809.57 + 1.587x	<0.001	0.057

[1] SEM: Standard error mean, [2] L: linear, [3] Q:quadratic.

Table 5. Apparent digestibility of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), non-fibrous carbohydrates (NFC), and total carbohydrates (TC) of rams fed.

Variable (gDM)	Cottonseed Lint (g/kg DM)				SEM [1]	Regression Equation	p-Value	
	0	70	140	210			L [2]	Q [3]
DM	707.45	690.42	638.82	643.15	11.453	Y = 706.63 − 3.493x	0.015	0.602
CP	609.27	548.29	416.78	335.84	25.461	Y = 620.32 − 13.597x	<0.001	0.486
NDF	330.68	348.47	421.55	483.63	17.070	Y = 316.30 + 7.599x	<0.001	0.315
EE	895.75	881.49	840.24	799.19	10.556	Y = 903.80 − 4.727x	<0.001	0.325
NFC	883.38	864.06	816.21	783.58	11.073	Y = 888.89 − 4.961x	<0.001	0.642
TC	694.88	721.62	672.24	680.59	14.487	Y = 692.33	0.504	0.764

[1] SEM: Standard error mean, [2] L: linear, [3] Q:quadratic.

3.3. Feeding Behaviour

The ingestive behavior presented an increasing linear effect ($p < 0.0001$) with the addition of lint for the intake time. The DM intake rate indicated a decreasing linear effect ($p < 0.0001$), similar to that observed for the rumination time ($p < 0.0001$) and for the DM rumination rate ($p < 0.001$) (Table 6).

3.4. Nitrogen Fractions

The intake of total nitrogen, digested nitrogen, and nitrogen balance were reduced linearly ($p < 0.01$) with the addition of lint in the diets; however, the addition of lint did not affect ($p = 0.194$) the fecal and urinary nitrogen (Table 7). The plasmatic ureic nitrogen was not affected ($p = 0.172$) by the addition of lint; however, the blood glucose was reduced linearly ($p = 0.036$) with the addition of lint (Table 7).

Table 6. Ingestive behavior of rams fed with cottonseed lint inclusion levels diets.

Variable	Cottonseed Lint (g/kg DM)				SEM [1]	Regression Equation	*p*-Value	
	0	70	140	210			L [2]	Q [3]
Chewing number/bit	70.60	60.32	66.65	58.01	2.138	Y = 63.90	0.093	0.838
Time chewing/bit	44.93	40.27	48.71	43.00	1.315	Y = 44.23	0.809	0.830
Intake time (min)	375.37	416.44	453.70	476.30	8.901	Y = 379.25 + 0.4901x	<0.001	0.063
IRDM (gDM/h)	175.03	145.24	96.27	64.73	9.801	Y = 177.30 − 0.5427x	<0.001	0.412
IRNDF (gNDF/h)	33.95	31.23	28.97	22.89	2.112	Y = 29.26	0.073	0.689
Rumination (min)	333.23	299.91	274.08	254.06	7.618	Y = 329.82 − 0.3762x	<0.001	0.388
RRDM (gDM/h)	198.44	177.28	149.23	139.77	9.292	Y = 196.79 − 0.2915x	0.014	0.874
RRNDF (gNDF/h)	43.16	49.29	46.25	49.52	4.965	Y = 47.06	0.743	0.896
Idle (min)	731.40	723.75	710.22	709.64	8.032	Y = 718.98	0.272	0.888

[1] SEM: Sandard error mean, [2] L: linear, [3] Q:quadratic, IRDM—intake rate of dry matter; IRNDF—intake rate of neutral detergent fiber; RRMS—rumination rate of dry matter; RRNDF—rumination rate of neutral detergent fiber.

Table 7. Average values of values of the nitrogen fractions in urine, feces, and plasma for diets containing cottonseed lint levels.

Variable	Cottonseed Lint (g/kg DM)				SEM [1]	Regression Equation	*p*-Value	
	0	70	140	210			L [2]	Q [3]
[4] IN	14.65	11.61	8.90	6.24	0.950	$\hat{Y}$ = 47.917 − 0.1293x	<0.001	0.486
[5] FN	7.96	5.27	5.51	4.48	0.670	$\hat{Y}$ = 10.88	0.541	0.194
[6] UN	2.16	1.71	1.79	1.78	0.076	$\hat{Y}$ = 26.186 − 0.0631x	<0.001	0.876
[7] DIGN	6.70	6.34	3.38	1.76	0.549	$\hat{Y}$ = 7.355+ 0.0341x	<0.001	0.043
[8] NB	4.54	4.63	1.59	−0.02	0.550	$\hat{Y}$ = 5.193 – 0.0239x	<0.001	0.223
[9] PUN	20.44	25.22	28.72	19.88	2.339	$\hat{Y}$ = 23.57	0.933	0.172
[10] Glucose	79.20	76.80	62.00	61.62	3.548	$\hat{Y}$ = 80.036 – 0.0965x	0.036	0.880

[1] SEM—standard error mean; [2] L—linear; [3] Q—quadratic; [4] IN—intake nitrogen (g/day); [5] FN—fecal nitrogen (g/day); [6] UN—urinary nitrogen (g/day); [7] DIGN—digested nitrogen (g/day); [8] NB—nitrogen balance(g/day); [9] PUN—plasmatic ureic nitrogen (mg/dL); [10] glucose—blood glucose (mg/dL).

4. Discussion

For every 10 g of lint added per kg of DM in the diet, there was a decrease of 2.83 g of CP consumed. According to the NRC [12], lambs with an average weight of 30 kg require an intake of 1.03 kg/day of DM and 97 g/day of CP, but all treatments were below the recommended, where intakes of DM were 986.56 g, 796.68 g, 722.31 g, and 517.81 g, and of CP g/day were 91.58, 72.59, 55.61, and 39.10, respectively. The addition of lint reduced the DM intake and hence the various bromatological fractions, except for the NDF due to the selectivity of animals that started to consume more hay than concentrate when the lint was added. The animals' selectivity can be evidenced through the diet effectively consumed (Table 4).

This result may be a consequence of the industrial extraction process of the lint since sulphuric acid is used and may generate residue in the final product (lint) [4]. This factor can be considered highly relevant in terms of acceptability because it is known that the odor, texture, color, and taste possibly affect the feed intake by animals. Nevertheless, Mertens [24] reported that acceptability is defined as the characteristic of feed associated with taste, olfactory, and visual activities that influence the intake of animals. There was no difference for the NDF intake; however, considering the diet effectively consumed as the ratio between the amount of NDF and DM consumed, and the NDF of the diet effectively consumed was linearly affected. It increased with the addition of lint, in which corn silage had a greater amount of this fraction (Table 1). The lint is also a source of NDF and ADF. Thus, the addition of lint promoted an increase of NDF in the concentrate, i.e., the animals received a diet with a higher content of NDF, and reduced NFC and EE.

The decrease of the apparent digestibility of DM, CP, EE, and NFC with the addition of lint may be related to the quality of the diet effectively ingested (Table 4). The composition of the fractions DM, CP, and TC of the corn grain, primarily observed in fractions A + B_1 (Table 2), are superior to those of the lint. Therefore, with the increased addition of lint, the animals started to eat less feed and of lesser quality, thus precluding the multiplication of the ruminal microorganisms, primarily reducing the apparent digestibility of the fractions cited above. Thereby, the rumen environment had a lower pH reduction, which apparently seems to have provided a more suitable environment for the development of fibrolytic bacteria, and thus a higher digestibility rate of the NDF, as observed by Santos et al. [25]. The treatment for extraction of lint uses sulphuric acid, which weakens the ether bonds between lignin and cellulose, making cellulose more digestible in the rumen, but with a slow degradation rate [26–28]. The data related to the NDF degradation of corn grain was higher than lint; however, the addition of lint decreased the degradation of other fractions due to the higher concentration of the undegradable fraction (C) of protein and carbohydrates, in addition to lower degradation of the DM and CP (Table 2), corroborating the linear reduction of digestibility with the addition of lint (Table 5).

Diets with the addition of lint promoted an increase in unavailable fractions of CP, such as neutral detergent insoluble nitrogen (NDIN). This fraction of CP is adhered to the plant cell wall and is generally indigestible. Thus, the CP digestibility is influenced by the increase of this fraction [29,30]. This fact is evidenced by observing the fractionation of CP in which the values for the fraction C (Table 2), which is undegradable, was higher in cotton lint compared to corn grain.

The apparent digestibility of EE decreased 4.73 g/kg with the addition of each 1 g/kg DM addition of lint in the diet (Table 5). The EE digestibility followed the decreasing linear effect of DM. The NFC digestibility was linearly reduced. This was possibly due to the smaller ruminal microbial population likely a function of the lower availability of CP and lack of synchrony between the availability of CP and energy [31].

There was no effect for the digestibility of TC as lint was added to the diet. This lack of effect is due to the increasing linear effect on the digestibility of NDF and decreasing linear effect on the digestibility of NFC. As TC is the sum of these fractions, the sum of these antagonistic behavior effects promotes the lack of effect by the addition of lint.

The ingestion time of the diet was likely negatively influenced by the reduced palatability of the diet with the addition of lint. Therefore, the animals went more often to the trough to eat, trying to consume the amount of diet sufficient to satisfy their requirements. However, even increasing the ingestion time, the animals were unable to compensate such demand, since they reduced the consumption of DM with the addition of lint (Table 6). The combined effect of reduced DM intake with increased ingestion time resulted in a reduced DM intake rate; the animals demonstrated a lower efficiency in DM intake. This effect was not observed in the NDF intake, due the increasing content of NDF observed with the addition of lint (Table 6), likely because of the lower rate of DM disappearance in the rumen and lower rate of passage.

Rumination time was affected in a linear decreasing way by the addition of lint despite an increase in NDF intake, which likely led to an increasing effect of rumination time. Despite it being emphasised in the literature that only physically effective NDF would have this effect, fibers from lint do not have the physical effect due to their reduced length [30].

The NB decreased with the addition of lint in the diet (Table 7), a factor that may be associated with a lower DM intake and the decrease of CP effectively consumed in the diets when lint was added. According to the NRC [12], the NB allows assessing the nutritional quality of diets and food, since it expresses how much of the absorbed nitrogen was used for animal metabolism [32,33]. The addition of lint in 210 g/kg DM promoted a negative NB, associated with the linear reduction in blood glucose provided by the addition of lint. Thus, it can be inferred that the addition of lint will promote a greater mobilization of the animal's body reserves, culminating in weight loss.

The addition of lint reduced the intake of concentrate (Table 4), which likely promoted a smaller rumen microbial population associated with a lower production of SCFA, primarily propionic acid, which is a glucose precursor via gluconeogenesis. Generally, glucose is not variable in ruminants, but when the animals are in a negative balance due to insufficient feed intake, blood glucose is altered, indicating a negative energy balance. This biological signalling is generally mediated by several signals that stimulate the mobilization of body energy reserves [33]. However, if inclusion in values up to 70 g in the diet for a short period of time is used, we may not have deleterious effects on the physiological parameters of the animals. Considering that the performance of the animals was not evaluated, it is possible that the inclusion at this level in short-term feeding may be a possibility to improve feed conversion [34]. The presence of lint can modulate the rate of passage and disappearance of food particles in the rumen.

According to Cavalcante et al. [35], the concentration in the diet or the differentiated intake of DM may cause variations in nitrogen intake [36]. In this research, the variation was to the detriment of the decreasing effect of DM intake. Another possible explanation was the reduced concentration of CP in the diet effectively consumed when lint was added. Although they were formulated to be isoproteined, there were variations in the consumption of the diets (Table 4). The glucose concentration reduced 0.96 mg/dL for each 1 g/kg DM of lint added in the diet, indicating a lower energy supply for the animals that received lint, a factor that may be related to the lower nutrient consumption when the by-product was added. However, even with the reduction, these values were within the reference range for sheep, which is 50–80 mg/dL [37].

The PUN concentration had no significant effect as the lint was added to the diet, with an average of 23.57 mg/dL, (Table 7). Menezes et al. [38] describes that the range in plasma urea concentration considered ideal for sheep is from 20.0 to 60.0 mg/dL, which was observed in this experiment. Lira et al. [39] reported that the plasma urea concentration is related to the part of digestible nitrogen that will be directed or be excreted via urine, or will be reused via saliva, increasing the efficiency of use of dietary nitrogen.

According to Gonzáles and Scheffer [40], the liver synthesizes urea from ammonia derived from the metabolism of amino acids and the absorption of rumen ammonia from protein degradation in the rumen, being directed through the blood for renal or mammary excretion when there is excess protein in the diet or even recycled via saliva when the diet has CP levels below requirements.

The addition of lint promoted a linear reduction in almost all parameters related to the use of the diet, even modifying the animals' ingestive behavior. The bromatological composition of lint suggests that this by-product can be tested as a source of roughage in the future.

5. Conclusions

The addition of up to 70 g/kg of cotton lint can be included in the diet of rams without adversely affecting DM intake and without altering digestibility, nitrogen balance, plasma urea, and glucose concentrations. These results suggest a possible negative effect on dry matter intake when using levels above 70 g/kg of cotton lint.

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