

Nitrogen Sources in Spineless Cactus-Based Diets for Sheep in Finishing

Lucíola Vilarim Ferraz

UFRPE: Universidade Federal Rural de Pernambuco

Ricardo Alexandre Silva Pessoa

UFRPE: Universidade Federal Rural de Pernambuco

Tarlan Oliveira Milanês

UFRPE: Universidade Federal Rural de Pernambuco

Maria Inês Sucupira Maciel

UFRPE: Universidade Federal Rural de Pernambuco

Juraci Marcos Alves Suassuna

UFPB: Universidade Federal da Paraíba

Kelly Cristina dos Santos

UFRPE: Universidade Federal Rural de Pernambuco

Daniel Barros Cardoso

UFRPE: Universidade Federal Rural de Pernambuco

Dorgival Moraes de Lima Júnior (✉ juniorzootec@yahoo.com.br)

Universidade Federal de Alagoas/Campus Arapiraca <https://orcid.org/0000-0002-1154-8579>

Antonia Sherlânea Chaves Vêras

UFRPE: Universidade Federal Rural de Pernambuco

Francisco Fernando Ramos de Carvalho

UFRPE: Universidade Federal Rural de Pernambuco

Research Article

Keywords: cotton by-product, feedlot, Nopalea, urea

Posted Date: June 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-547192/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

This study evaluated the effect of different sources of nitrogen [soybean meal (SM); cottonseed meal (CM); cottonseed + urea (CU); and urea (U)] associated with spineless cactus on intake, digestibility, performance, carcass characteristics and meat quality of confined lambs. Forty male Santa Inês sheep, uncastrated, with initial body weight of 23.00 ± 1.66 kg were distributed in random blocks design and slaughtered after 58 days of confinement. Dry matter (DM) intake (1.16 ± 0.19 kg/day) did not differ ($P > 0.05$) between nitrogen sources, but the intake of crude protein (CP) and non-fibrous carbohydrates (NFC) was lower ($P < 0.05$) for sheep consuming CU. Sheep fed with diets containing U had lower digestibility of DM, CP and NFC ($P < 0.05$). The average daily gain (0.15 ± 0.04 kg/day) and the slaughter body weight (32.07 ± 2.86 kg) did not differ between nitrogen sources, but the cold carcass and loin weight was lower ($P < 0.05$) for sheep fed with U. The water retention capacity and meat protein content were greater ($P < 0.05$) for animals fed with SM. Soybean meal or cottonseed meal associated with spineless cactus-based diets for sheep meat production are recommended because it allows greater DM digestibility and improves carcass characteristics and physicochemical composition of the meat.

Introduction

Spineless cactus has been consolidated as a food rich in high fermentation carbohydrates for ruminant diets in arid and semi-arid areas of the planet, because in addition to being an energy source, it is an important water fountain for livestock production (Albuquerque et al., 2020; Moura et al., 2020; Rocha-Filho et al., 2021). This cactus has high levels of non-fibrous carbohydrates (432.62 ± 15.0 g/kg DM) divided into starches, pectins and soluble sugars (Sáenz et al., 2004; Batista et al., 2009; Pessoa et al., 2020). However, low levels of crude protein (46.15 ± 2.1 g/kg DM) and neutral detergent fiber (326.46 ± 12.1 g/kg DM) (Santos et al., 2018) indicate the need to associate this roughage with sources of nitrogen (N) and fiber in the diet of ruminants.

Despite establishing the appropriate fiber level for diets with spineless cactus (Vieira et al., 2008; Pinho et al., 2018), there is no recommendation of which N source to use in complete diets with predominance of spineless cactus. However, for the optimal growth of the ruminal microorganisms is crucial the association the energetic ingredients with an N source. Especially if we consider the need for synchronism between the degradation rates of carbohydrate sources - spineless cactus with 733 g/kg carbohydrates in fraction A + B1 - and N in the animals' rumen.

Soybean meal is the N sources with rapid ruminal degradation traditionally used in ruminant feed, but in semi-arid areas the production difficulty and market competition results in high prices. Regarding this, Santos et al. (2020a) suggest that diets containing spineless cactus associated with urea can reduce freshwater intake and allow reasonable body weight gain. The cottonseed, in turn, can be both a source of fiber as the N in diets with spineless cactus.

Therefore, using other N sources at a lower cost could improve the viability of livestock production systems, especially in semi-arid regions, and thus enable a greater supply of sheep meat. Given the above, the objective was to evaluate the potential use of different protein sources associated with spineless cactus (*Nopalea cochenillifera* Salm Dyck) in diets for finishing lambs, by determining the performance and characteristics of the carcass and meat.

Material And Methods

The experiment was carried out at the Department of Animal Science of the Federal Rural University of Pernambuco (UFRPE), located in Recife-PE, Brazil. The climate is classified, according to Koppen, as being of the Ams' type, which is characterized by being hot and humid, with an average annual temperature of 25.2°C.

Animals, management and diets

Forty male Santa Inês sheep, uncastrated, with an average body weight of 23 kg \pm 1.66 were identified and treated against ecto and endoparasites and, fasted for 16 hours, being weighed and distributed in a randomized block design, with two blocks, four treatments and ten repetitions. The animals were housed in individual stalls (1.0 m x 1.8 m), provided with individual feeder and drinker. The experimental period comprised 86 days, 28 days of which were used to adapt the animals to the facilities and handling, and 58 to the period of data and sample collection.

The experimental treatments consisted of four different sources of nitrogen (soybean meal (SM); cottonseed meal (CM); cottonseed + urea (CU); and urea (U)), with roughage composed of Tifton 85 grass hay (*Cynodon* spp.) and spineless cactus (*Nopalea cochenillifera* Salm Dyck), as shown in Table 1. Rations were calculated to meet weight gains of 200 g/day (NRC, 2007).

Table 1
Participation of ingredients and chemical composition of experimental diets

Ingredients (g/Kg DM)	Nitrogen sources			
	Soybean meal	Cottonseed meal	Cottonseed + urea	Urea
Spineless cactus	460.0	450.0	440.0	410.0
Ground corn	70.0	100.0	44.0	244.0
Tifton hay	300.0	260.0	224.0	300.0
Soybean meal	150.0	-	-	-
Cottonseed meal	-	170.0	-	-
Cottonseed	-	-	270.0	-
Urea/Ammonium sulfate	-	-	12.0	23.0
Vegetable oil	10.0	10.0	-	10.0
Limestone	5.0	5.0	5.0	3.0
Mineral mix	5.0	5.0	5.0	10.0
<i>Chemical composition</i>				
Dry matter (g/kg)	232.20	236.40	240.85	252.87
Organic matter (g/kg DM)	878.83	881.79	885.78	867.89
Crude protein (g/kg DM)	114.30	138.12	127.46	118.53
Ether extract (g/kg DM)	27.91	28.76	58.65	34.39
Neutral detergent fiber ap (g/kg DM)*	406.60	392.24	451.43	387.57
Acid detergent fiber (g/kg DM)	203.93	193.20	254.43	190.09
Non-fibrous carbohydrates (g/kg DM)	344.60	336.71	283.32	402.39
Total digestible nutrients (g/kg DM)	607.80	603.90	664.83	554.55
*Neutral detergent fiber corrected for ash and protein.				

The spineless cactus, composed mostly of cladodes, was processed in a forage machine, immediately before supply; Tifton 85 grass hay ground in a forage machine with a 4 mm sieve; corn and cottonseed meal were ground; while the cottonseed, whole. Commercial urea and ammonium sulfate used was Petrobras® (Petrobras, SE, Brazil). The diet was provided as a complete mixture, twice a day (8 am and 3

pm), with 60% of the diet offered in the morning and 40% in the afternoon. The adjustment of the quantity offered was carried out every two days, in order to allow 15% of leftovers.

Nutrient intake and apparent digestibility

The amount of feed offered and the leftovers were weighed daily to calculate the voluntary intake. Nutrients intake was calculated by the difference between the nutrient concentration in supplied feed and nutrient concentration in the leftovers. For the apparent digestibility test, feces samples were collected, directly from the rectal ampoule of the animals, for six days at 0; 2; 4; 6; 8; and 10 hours after feeding. The feces samples were homogenized, pre-dried in a forced ventilation oven at 55°C for 72 hours and, later, processed in a sieve mill with a 2 mm sieve for future analysis.

The estimation of fecal dry matter production (FDMP) was performed using indigestible neutral detergent fiber (iNDF) as an internal indicator, obtained after an incubation period of 288-h in bovine with a permanent cannula in the rumen, according described by Valente et al. (2011). The FDMP was then estimated by the relationship between the intake of the marker and the respective percentage in the feces. The digestibility of dry matter and nutrients was calculated by the relationship between the difference between the amount of dry matter or nutrient ingested and that excreted, by the amount of dry matter or nutrient ingested, and expressed in g/kg DM.

Chemical composition

During the experimental period, samples of the leftovers, feces and the ingredients used were collected, which were pre-dried for 72 hours in a forced ventilation oven at 55°C, identified and stored. At the end of the experiment, a sample was made up of leftovers from the entire experimental period, per animal, as well as the food supplied, which were processed in a mill with 1 mm diameter sieve screens, for further bromatological analysis.

The determinations of DM (method 930.15); ash (method 942.05), crude protein (CP) (method 968.06) and ether extract (EE) (method 954.05), were performed according to AOAC (2012). The levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methodology described by Van Soest et al. (1991). The correction of the NDF for ash and protein (NDFap) was performed using the methodologies for CP and ash. To estimate total carbohydrates (TC), the equation proposed by Sniffen et al. (1992): $\%TC = 100 - (\%CP + \%EE + \%ash)$ and, due to the presence of urea in the diets, the levels of non-fibrous carbohydrates (NFC) were calculated as proposed by Hall (2001), being: $NFC = 100 - [(\%CP - \%CP \text{ derived from urea} + \%urea) + \%NDFap + \%EE + \%ash]$.

Ingestive behavior

The behavioral parameters were observed using the instantaneous scanning method ("Scan sampling"), proposed by Martin and Bateson (2007), at intervals of five minutes, for 24 hours (Johnson and Combs, 1991). In the observation intervals, the variables were determined: feeding, rumination and idling times. The total chewing time (TCT, min/day) was considered as the sum of the feeding and rumination times (FT + RUT) and the idle time, as the time when the animal was neither feeding nor ruminating. Feeding

efficiency as a function of DM intake (FE_{DM} , g DM/min) was calculated as the relationship between DM consumption and feeding time (DMI/FT); and as a function of the NDF intake (FE_{NDF} , g NDF/min) as the fraction of the NDF intake, by the feeding time (NDFI/FT). The rumination efficiencies as a function of DM intake (RUE_{DM} , g DM/min) and NDF intake (RUE_{NDF} , g NDF/min), were obtained respectively through the relationships between DM intake (DMI/RU) and NDF (NDFI/RU), and the rumination time.

Performance, slaughter and meat quality

During the experimental period, the animals were weighed without fasting every 14 days, to monitor development without compromising performance. The average daily gain (ADG) was calculated according to the final and initial weights, with a 16-hour fast.

After completing the 58 days of data and sample collection, the animals were subjected to fasting solids for 16 hours. After this time, they were weighed to obtain body weight at slaughter (BWS). Slaughter operations were carried out in accordance with the current rules of RIISPOA (Brasil, 2000). Initially the animals were desensitized by the non-penetrative percussive method, suspended by the hind limbs attached to hooks, followed by bleeding from the carotid arteries and jugular veins.

Skinning and evisceration was performed head and paws were removed to record the hot carcass weight (HCW), including kidneys and pelvic-renal fat. The gastrointestinal tract was weighed filled and then emptied, washed and weighed again, to determine of the empty body weight (EBW), obtained by the difference between the BWS and the content of the gastrointestinal tract (CGIT).

Afterwards, the hot carcasses were taken to the cold chamber and kept for 24 hours at a temperature of 4°C, with the tarsus-metatarsal joints approximately 14 cm apart, using their own hooks. Then, the cold carcass weight (CCW) was recorded, including kidneys and pelvic-renal fat; weight by cooling loss (CL = HCW – CCW) was calculated and kidney weights and pelvic-renal fat were obtained, whose values were subtracted to determine the hot (HCW) and cold (CCW) carcass weights; as well as hot carcass yields (HCY (%) = $HCW/BWS \times 100$) and cold carcass (CCY (%) = $CCW/BWS \times 100$).

In addition, after the cooling period, objective measurements were performed on the entire carcass, according to Cezar and Sousa (2007). From the establishment of the relationships between the measures cold carcass weight, internal carcass length, croup width and leg length, the carcass compactness indexes (CCI (kg/cm)) were calculated.

After weighing and removing the tails, the carcasses were divided sagittally and the half carcasses were divided into six anatomical regions that constituted the meat cuts: leg, loin, rib, saw, neck, and shoulder (Cezar and Sousa, 2007).

The *Longissimus* muscle area (LMA) was measured in the left half carcass, through a cross section between the 12th and 13th ribs, exposing the cross section of the *Longissimus dorsi* muscle, through its outline, using transparent plastic sheet. To read the LMA, a digital planimeter (HAFF®; model Digiplan)

was used. Subcutaneous thickness fat (STF) was also measured in *Longissimus lumborum*, using a digital caliper, according to the method described by Cesar and Sousa (2007).

To determine tissue composition, the left legs were dissected according to the methodology described by Cesar and Souza (2007). With the aid of a scalpel and tweezers, subcutaneous fat, intermuscular fat, muscles, bones and other tissues (tendons, lymph nodes, nerves and blood vessels) were removed. During dissection, the top five muscles that surround the femur (*Bicepsfemoris*, *Semimembranosus*, *Semitendinosus*, *Quadricepsfemoris* and *Adductor*) were removed in their entirety and, with the measurement of the length of the femur (cm), the leg muscle index (LMI) was calculated using the formula proposed by Purchas et al. (1991).

After dissecting the legs, samples of the *Semimembranosus* muscle were crushed in a blender to obtain a homogeneous paste, and then they were lyophilized to determine moisture (Method n. 925.04), crude protein (Method n. 981.10), ether extract (Method n. 935.38) and ashes (Method 938.08), according to AOAC (2000).

Samples of the *Longissimus lumborum* muscle were used for the physical-chemical analysis of the meat. The chromatic characterization was performed according to Ramos and Gomide (2009) using Minolta colorimeter, model Chroma Meter CR-400, operating in the CIE system (L^* , a^* , b^*). The water-holding capacity (WHC) was determined according to the methodology described by Sierra (1986). For pH, 10g samples were collected, crushed and diluted with 150 ml of distilled water and agitated until the particles were evenly suspended, and the reading was performed with the aid of a potentiometer (Gomes and Oliveira, 2011). Cooking loss (CL) was determined according to the procedure cited by Duckett et al. (1998).

Statistical analysis

The experimental design was randomized blocks, with the initial weight of the animals being the criterion for block formation, according to the following model:

$$Y_{ij} = \mu + T_i + b_j + e_{ij}$$

where Y_{ij} = the observed value of the dependent variable, μ = the overall average, the treatment effect = ($i = 1$ to 4), b_j = the effect of block j ($j = 1$ to 4) and e_{ij} = the experimental error. Data were analyzed using analysis of variance (ANOVA), using the GLM procedure and SNK test ($P < 0.05$), using the statistical package Statistical Analysis System (SAS (Statistical Analysis Systems Institute Inc.), 2000).

Results

The nitrogen sources tested did not interfere ($P > 0.05$) on the DM and OM intake of the sheep, but the CP intake and NFC was lower ($P < 0.05$) for animals fed with cottonseed + urea (CU) followed by urea (U) (Table 2). The NDF intake was lower ($P < 0.05$) for the soybean meal (SM) diet, but similar between the

cottonseed meal (CM), CU and U diets. While the EE intake was higher ($P < 0.05$) for lambs fed SM. The average consumption of TDN was 0.71 kg/day and did not differ ($P > 0.05$) between diets.

Table 2
Intake and apparent digestibility of nutrients by sheep fed different nitrogen sources in diets based on spineless cactus

Item	Nitrogen sources				SDM	<i>P-Value</i>
	Soybean meal	Cottonseed meal	Cottonseed + urea	Urea		
<i>Intake (kg/day)</i>						
Dry matter	1.19	1.28	1.07	1.11	0.192	> 0.05
Organic matter	1.07	1.14	0.95	1.00	0.171	> 0.05
Crude protein	0.17 ^a	0.17 ^a	0.11 ^c	0.14 ^b	0.027	< 0.01
Ether extract	0.04 ^a	0.03 ^b	0.03 ^b	0.03 ^b	0.007	< 0.01
Neutral detergent fiber	0.40 ^b	0.51 ^a	0.47 ^{ab}	0.43 ^{ab}	0.082	0.03
Non-fibrous carbohydrates	0.46 ^a	0.44 ^a	0.37 ^b	0.47 ^a	0.069	0.01
Total digestible nutrients	0.77	0.77	0.68	0.62	0.150	> 0.05
<i>Digestibility (g/kg)</i>						
Dry matter	561 ^a	588 ^a	606 ^a	448 ^b	55.10	< 0.01
Organic matter	611 ^a	629 ^a	635 ^a	507 ^b	50.20	< 0.01
Crude protein	622 ^{ab}	652 ^a	559 ^{ab}	530 ^b	83.58	< 0.01
Ether extract	787 ^a	722 ^a	700 ^{ab}	614 ^b	88.52	< 0.01
Neutral detergent fiber	421 ^{bc}	453 ^{ab}	543 ^a	337 ^c	77.78	< 0.01
Non-fibrous carbohydrates	775 ^{ab}	834 ^a	814 ^a	735 ^b	52.61	< 0.01
SDM = Standard deviation from mean; Means with a different letter on the same line differ from each other by the SNK test ($p < 0.05$).						

The sheep fed SM diet had shorter ($P < 0.05$) feeding, rumination and chewing times and longer ($P < 0.05$) idle times concerning to animals fed with other nitrogen sources (Table 3). Better efficiency of feeding and rumination of dry matter ($P < 0.05$) were also observed in animals fed with SM.

Table 3
Ingestive behavior of sheep fed different nitrogen sources in diets based on spineless cactus

Item	Nitrogen sources				SDM	<i>P</i> -value
	Soybean meal	Cottonseed meal	Cottonseed + urea	Urea		
Feeding time (min)	147.5 ^b	217.0 ^a	198.5 ^a	224.5 ^a	51.24	< 0.01
Rumination time (min)	410.0 ^b	523.5 ^a	498.5 ^a	506.0 ^a	81.05	0.02
Idle time (min)	882.5 ^a	699.5 ^b	752.0 ^b	709.5 ^b	95.94	< 0.01
Chewing time (min)	557.5 ^b	740.5 ^a	688.0 ^a	730.5 ^a	95.89	< 0.01
Feeding efficiency (g DM/min)	8.71 ^a	5.94 ^b	5.76 ^b	5.38 ^b	2.05	< 0.01
Feeding efficiency (g NDF/min)	2.93	2.38	2.58	2.11	0.758	> 0.05
Rumination efficiency (g DM/min)	3.12 ^a	2.46 ^{ab}	2.20 ^b	2.26 ^b	0.667	0.01
Rumination efficiency (g NDF/min)	1.06	0.98	0.98	0.88	0.249	> 0.05
SDM = Standard deviation from mean; Means with a different letter on the same line differ from each other by the SNK test ($p < 0.05$).						

The nitrogen sources did not influence ($P > 0.05$) the final body weight (32.07 kg), average daily gain (0.15 kg/day) and feed conversion (7.73) of the sheep (Table 4).

Table 4
Performance of sheep fed different nitrogen sources in diets based on spineless cactus

Item	Nitrogen sources				SDM	<i>P</i> -value
	Soybean meal	Cottonseed meal	Cottonseed + urea	Urea		
Initial body weight (kg)	23.19	23.71	22.89	22.01	1.44	-
Final body weight (kg)	33.71	32.85	30.81	30.92	2.86	> 0.05
Average daily gain (kg/d)	0.18	0.16	0.14	0.15	0.04	> 0.05
Total weight gain (kg)	10.54	9.14	7.94	8.91	2.55	> 0.05
Food conversion	6.85	8.42	7.98	7.67	1.44	> 0.05
SDM = Standard deviation from mean; Means with a different letter on the same line differ from each other by the SNK test ($p < 0.05$).						

The weight of the empty body was not influenced ($P > 0.05$) by the nitrogen sources tested, but the weight of the cold carcass was higher ($P < 0.05$) for sheep fed with SM and CM when compared to animals fed with U (Table 5).

Table 5

Carcass characteristics and meat cuts of sheep fed different nitrogen sources in diets based on spineless cactus

Item	Nitrogen sources				SDM	P-value
	Soybean meal	Cottonseed meal	Cottonseed + urea	Urea		
Body weight at slaughter (kg)	33.71	32.85	30.81	30.92	2.86	> 0.05
GITC ¹ (kg)	1.54	1.16	1.49	1.60	1.06	> 0.05
Empty body weight (kg)	32.17	31.69	29.32	29.32	3.02	> 0.05
Biological yield (%)	49.00	48.83	49.68	47.48	2.61	> 0.05
Cold carcass weight (kg)	15.17 ^a	14.85 ^a	13.89 ^{ab}	13.42 ^b	1.27	0.013
Cold carcass yield (%)	45.05	45.22	45.03	43.46	1.75	> 0.05
Cooling losses (%)	3.29	3.94	4.70	3.36	1.38	> 0.05
Internal carcass length (cm)	59.20	59.10	58.40	58.10	2.22	> 0.05
Thoracic perimeter (cm)	69.30 ^a	68.90 ^{ab}	67.43 ^{ab}	66.50 ^b	2.19	0.025
Carcass compactness index (kg/cm)	0.26 ^a	0.25 ^a	0.24 ^{ab}	0.23 ^b	0.01	0.019
<i>Longissimus</i> muscle area (cm ²)	10.92	11.09	10.32	9.98	1.44	> 0.05
Subcutaneous fat thickness (mm)	1.34	1.09	1.20	1.24	0.32	> 0.05
Perirenal fat score	2.50	2.40	2.40	2.40	0.52	> 0.05
Leg (kg)	2.29	2.27	2.12	2.06	0.19	> 0.05
Shoulder (kg)	1.32 ^a	1.28 ^{ab}	1.21 ^b	1.18 ^b	0.10	0.014
Rib (kg)	1.14	1.19	1.11	1.05	0.12	> 0.05

¹Gastrointestinal tract content. SDM = Standard deviation from mean; Means with a different letter on the same line differ from each other by the SNK test (p < 0.05).

Item	Nitrogen sources				SDM	<i>P</i> -value
	Soybean meal	Cottonseed meal	Cottonseed + urea	Urea		
Saw (kg)	1.08	1.11	1.04	0.99	0.16	> 0.05
Neck (kg)	0.86	0.83	0.78	0.78	0.10	> 0.05
Loin (kg)	0.78 ^a	0.76 ^a	0.68 ^{ab}	0.64 ^b	0.10	0.016

¹Gastrointestinal tract content. SDM = Standard deviation from mean; Means with a different letter on the same line differ from each other by the SNK test ($p < 0.05$).

The carcass length was similar ($P > 0.05$) between the diets, however the thoracic perimeter and the carcass compactness were higher ($P < 0.05$) in the carcasses of sheep fed with SM compared to U. The subcutaneous fat thickness and the pelvic-renal fat score did not differ ($P > 0.05$) between the nitrogen sources tested.

The weights of the shoulder and loin of the carcasses of the sheep fed with SM were higher ($P < 0.05$) than the animals fed with U. However, we did not observe an effect ($P > 0.05$) of the tested diets on the tissue composition and compactness index of the sheep leg (Table 6).

Table 6

Tissue composition of sheep legs fed different nitrogen sources in diets based on spineless cactus

Item	Nitrogen sources				SDM	<i>P</i> -value
	Soybean meal	Cottonseed meal	Cottonseed + urea	Urea		
Muscle (kg)	1.48	1.42	1.36	1.29	0.15	> 0.05
Bone (kg)	0.45	0.41	0.44	0.40	0.04	> 0.05
Fat (kg)	0.19	0.18	0.16	0.15	0.04	> 0.05
Other tissues (kg)	0.06	0.05	0.07	0.06	0.05	> 0.05
Muscle (%)	67.48	68.28	66.51	67.09	0.02	> 0.05
Bone (%)	20.88	20.16	21.94	20.96	2.65	> 0.05
Fat (%)	8.72	8.84	7.84	7.74	0.35	> 0.05
Leg muscle index	0.38	0.39	0.37	0.38	2.41	> 0.05

SDM = Standard deviation from mean; Means with a different letter on the same line differ from each other by the SNK test ($p < 0.05$).

The content of moisture, ash and ether extract of sheep meat did not differ ($P > 0.05$) between the nitrogen sources tested, but the protein content of meat from animals fed with SM was higher when compared to those fed with CM, COT and U (Table 7).

Table 7

Chemical composition and physicochemical parameters of meat (*Semimembranosus and Longissimus dorsi*) from sheep fed different nitrogen sources in diets based on spineless cactus

Item	Nitrogen sources				SDM	P-value
	Soybean meal	Cottonseed meal	Cottonseed + urea	Urea		
Moisture (%)	74.02	75.50	75.62	76.00	2.21	> 0.05
Ash (%)	1.26	1.27	1.48	1.59	0.37	> 0.05
Ether extract (%)	2.69	2.59	2.97	2.59	0.39	>0.05
Crude protein (%)	21.67 ^a	20.17 ^b	19.45 ^b	19.10 ^b	1.37	< 0.01
pH	5.51	5.38	5.44	5.33	0.14	> 0.05
L*	42.78	41.25	41.22	43.45	2.20	> 0.05
a*	14.55	14.05	14.21	14.66	1.33	> 0.05
b*	7.62	6.61	6.66	7.16	0.36	> 0.05
Cook losses (%)	37.81	37.56	39.15	37.54	4.28	> 0.05
Water-holding capacity (%)	34.72 ^a	29.58 ^{ab}	24.74 ^{bc}	20.03 ^c	6.68	< 0.01
SDM = Standard deviation from mean; Means with a different letter on the same line differ from each other by the SNK test ($p < 0.05$).						

Except for water-holding capacity, there was no influence ($P > 0.05$) of the nitrogen sources tested on the pH values, color parameters and cooking losses of the meat.

Discussion

The DM intake of sheep (average of 1.16 kg/day) was high when compared to that predicted by the NRC (2007) (0.96 kg/day) for the same animal category (28 kg and gain of 0.20 kg/day). Possibly, the spineless cactus in the diets optimized the intake of the animals, reducing the powderiness of the total

diet and facilitating the apprehension, by the animals, of the mixture in the trough. In a meta-analysis Knupp et al. (2019) reported that the level of 500 g/kg of spineless cactus in the dry matter of the sheep diet - a level close to that used in the present study - obtains the maximum diet intake by the animals.

The lowest intake of CP was observed for the diets CU (0.11 g/day) and U (0.14 g/day). This can be explained by the fact that the animals rejected part of the cottonseed in this diet - even with spineless cactus facilitating the mixing of the ingredients, the cottonseed was selected and despised by the animals - reflecting a lower CP intake than the concentration of this nutrient in the diet. As for the NDF intake, we observed that the animals fed with the SM diet consumed less NDF, probably due to the majority of the NDF of this diet coming from Tifton 85 hay, which was more selected by the animals due to the larger particle size in the total mixture. Costa et al. (2012) also observed a reduction in the intake of NDF and CP in sheep fed with cottonseed in diets based on spineless cactus.

The lower dietary levels of NFC observed in the CU diet possibly explain the lower consumption of NFC compared to other N sources. In addition, due the CU diet has a higher EE content, it was expected that the highest intakes of this nutrient would occur in animals submitted to this diet, a fact that did not occur. However, as previously mentioned, rejection of the cottonseed by the animals was observed during the experimental period.

The lower apparent digestibility coefficient of DM and OM for U diet, probably due to the higher NFC content derived from corn in this diet. We propose that the excess of NFC - and lower NDF content - provided a reduction in ruminal pH and decreased the microbial population degrading fibrous carbohydrates, resulting in less digestibility of DM in the total diet. In line with our findings, Santos et al. (2020b) observed that diets containing urea as a total substitute for soybean meal alter the production of ruminal short-chain fatty acids and reduce the populations of *Streptococcus bovis* and *Ruminococcus flavefaciens*.

The lower apparent digestibility of the CP for U concerning to SM and CM diets was possibly due to the greater synchronism between carbon and nitrogen. In the case of U-fed sheep, the rapid conversion of urea to ammonia and its immediate rumen absorption meant that the level of nitrogen available for microbial protein synthesis of U-fed sheep was lower than those fed with CM (Ahvenjärvi and Huhtanen, 2018).

The lower digestibility of NFC observed in the U diet is due to the nature of the NFC in this diet, which consists predominantly of corn starch. The corn starch *flint* has a slower degrabability compared to the NFC fraction present in spineless cactus (soluble sugars, starch and fruits) (Batista et al. 2003), resulting in less digestibility of the NFC fraction of the U diet compared to the other diets that had the forage palm as the main source of NFC. Still in this context, the lower digestibility of NDF observed in the U diet, is precisely due to the depressant effect of excess NFC on pH and NDF-degrading microbiota.

The SM diet showed greater efficiency of intake mainly due to the shorter feeding time, since the consumption of DM did not differ among nitrogen sources. Batista et al., (2020) observed an increase in

feeding time with the replacement of soybean meal with a mixture of cottonseed and spineless cactus for buffaloes. In addition, the lower NDF intake of animals fed with SM may explain the higher rumination efficiency observed in this experimental group.

The average daily gain of the sheep (0.15 kg/day) was similar between nitrogen sources, possibly due to the proximity in the TDN intake of the animals. Similarly, Shen et al. (2018) observed that the substitution of soybean meal for another source of nitrogen did not influence the final weight of lambs. It is worth mentioning that the diets were formulated for gains of 0.20 kg/day, but none of the treatments achieved the gain predicted in the NRC (2007). Possibly, differences in the genetic composition of animals (Oliveira et al., 2018) and in food - diets with spineless cactus - (Knupp et al., 2019) may help explain these differences between the predicted and observed gain.

The slaughter body weight and the empty body weight were similar between the nitrogen sources tested, probably due to the proximity in the average daily gain of the animals fed with the different nitrogen sources. However, the carcasses of sheep fed with SM and CM were about 12% heavier than the carcasses of sheep fed with U. Probably, the higher consumption of digestible organic matter observed for animals fed with SM (0.65 kg/day) and CM (0.71 kg/day) compared to U (0.51 kg/day) increased the deposition of muscle tissue in the carcass (see carcass compactness index) of sheep fed with SM and CM to the detriment of those fed with U. In this context, Sano et al. (2009) suggested that the addition of urea to the sheep diet may interfere with the synthesis of the animals' body protein, possibly by reducing the flow of amino acids to the small intestine of ruminants (Broderick & Raynal, 2009).

The lower weights for shoulder and loin obtained in the carcasses of sheep fed with U in concerning to SM and CM must be associated with the differences observed for the CCW between these diets. However, these differences were not sufficient to alter the deposition (kg or %) of muscle, fat and bone in the animals' carcasses. This results corroborates with Alves et al. (2016) that also observed a reduction in the weight of the sheep's shoulder and loin when soybean meal was replaced by other N sources in the diet. And, in contrast Atti & Mahouachi (2009) also did not observe differences in the composition (muscle, fat and bone) of the leg of lambs fed with different sources of nitrogen.

In addition to the fat in the carcass, the nitrogen sources tested did not influence the amount of fat in sheep meat. However, animals fed with SM produced meat that was richer in protein, influenced by the higher crude protein intake. Normally, the highest protein content in meat is associated with increases in myofibrillar and sarcoplasmic proteins that occur due to the increase in the contents of the muscle's DM. Because, the majority content of water is maintained by capillary forces within the myofibrils and these occupy about 82–87% of the volume of the muscle cell (Huff-Lonergan & Lonergan, 2005), and much as 85% of the water in a muscle cell is held in the myofibrils (Hughes et al., 2014), consequently, the meat of animals fed with SM showed a higher WHC value, this was due to the direct relationship between the water content of the muscle and content and type of protein of type, this may have occurred due to a lower solubility of the proteins present in the meat of lambs fed with SM, since the moisture content was similar between nitrogen sources. The inferior WHC can negatively affect the appearance of meat, and

this can influence consumer willingness to purchase the product (Hughes et al., 2014). In addition, the possible increase in sarcoplasmic proteins (myoglobin) would result in a reduction in L* values and increments in a*, responses that were also not observed.

Regarding the physical-chemical characteristics, the similarity observed for the values of L*, a*, b* and cooking losses of sheep meat may be related to the proximity in the final pH values of meat of sheep fed with different sources nitrogen. Silva et al. (2020) also did not observe the effect of replacement of soybean meal for urea on the a* values and cooking losses by the meat.

Soybean meal or cottonseed meal associated with spineless cactus-based diets for sheep meat production are recommended because it allows greater DM, OM, CP and EE digestibility, eating and ruminating efficiency rate, and improves carcass characteristics and physicochemical composition of the meat.

Declarations

Funding

The authors thank the Foundation for Research Support of the State of Pernambuco (FACEPE) and the National Council for Scientific and Technological Development (CNPq) for funding the research; and the Coordination of Improvement of Personnel for Higher Education (CAPES) for the master's degree scholarship.

Conflict of interest

The authors declare that there are no competing interests.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by L V Ferraz, T O Milanês, J M Suassuna, K C Santos and D B Cardoso. The first draft of the manuscript was written by R A Pessoa, M I S Maciel, D M Lima Júnior, A S C Vêras, F F R Carvalho and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors thank the Foundation for Research Support of the State of Pernambuco (FACEPE).

Ethics approval

All procedures were conducted in accordance with the guidelines set by the Brazilian College of Animal Experimentation and approved by the Ethics Committee on Use of Animal for Research (CEUA) of the Federal Rural University of Pernambuco (License 053/2015).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Albuquerque, I., Araújo, G., Santos, F., Carvalho, G., Santos, E., Nobre, I., Bezerra, L., Silva-Júnior, J., Silva-Filho, E., and Oliveira, R. 2020. Performance, Body Water Balance, Ingestive Behavior and Blood Metabolites in Goats Fed with Cactus Pear (*Opuntia ficus-indica* L. Miller) Silage Subjected to An Intermittent Water Supply. *Sustainability*, 12, 2881. doi:10.3390/su12072881
2. Alves, F. J. L., Ferreira, M. A., Urbano, S. A., Andrade, R. P. X., Silva, Á. E. M., Siqueira, M. C. B., Oliveira, J. P. F. and Silva, J. L. 2016. Performance of lambs fed alternative protein sources to soybean meal. *Revista Brasileira de Zootecnia*, 45, 145-150. <http://dx.doi.org/10.1590/S1806-92902016000400001>
3. Ahvenjärvi, S. and Huhtanen, P. 2018. Effects of intraruminal urea-nitrogen infusions on feed intake, nitrogen utilization, and milk yield in dairy cows. *Journal of Dairy Science*, 101, 9004–9015.
4. AOAC, Association of Official Analytical Chemists. 2000. *Official methods of analysis*. (17th ed.) Washington: AOAC.
5. Atti, N., and Mahouachi, M. 2009. Effects of feeding system and nitrogen source on lamb growth, meat characteristics and fatty acid composition. *Meat Science*, 81, 344–348. <https://doi.org/10.1016/j.meatsci.2008.08.011>
6. Batista, A. M. V., Mustafa, A. F., Santos, G. R. A., Carvalho, F. F. R., Dubeux Jr, J. C. B., Lira, M. A., and Barbosa, S. B. P. 2003. Chemical composition and ruminal dry matter and crude protein degradability of spineless cactus. *Journal Agronomy & Crop Science*, 189(2), 123–126. <https://doi.org/10.1046/j.1439-037X.2003.00008.x>
7. Batista, A. M. V., Ribeironeto, A. C., Lucena, R. B., Santos, D. C., Dubeux Jr, J. C., and Mustafa, A. F. 2009. Chemical Composition and Ruminal Degradability of Spineless Cactus Grown in Northeastern Brazil. *Rangeland Ecology & Management*, 62, 297–301. <https://doi.org/10.2111/07-099R1.1>
8. Batista, J. N., Pereira, F. B., Pereira Filho, J. M., Lima Junior, V., Santos, V. L. F., Araújo, M. J., Moura, J. P., Pereira, E. S., Oliveira, R. L., and Bezerra, L. R. 2020. Replacing corn bran and soybean meal in the

- diet with spineless cactus and cottonseed affects ingestive behaviour, performance, carcass characteristics and meat quality of Murrah water buffalo. *Animal Production Science*, 60, 903–912. <https://doi.org/10.1071/AN19260>
9. Brasil. 2000. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 3, de 07 de janeiro de 2000. Regulamento técnico de métodos de insensibilização para o abate humanitário de animais de açougue. SDA-MAPA. Diário Oficial da União, Brasília, p.14-16, 24 de janeiro de 2000, Seção I. Available in: <<http://extranet.agricultura.gov.br/sislegisconsulta/consultarLegislacao.do?operacao=visualizar&id=1793>> Access in: 03 Feb 2021.
 10. Broderick, G. A., and Reynal, S. M. 2009. Effect of source of rumen-degraded protein on production and ruminal metabolism in lactating dairy cows. *Journal of Dairy Science*, 92, 2822–2834. doi: 10.3168/jds.2008-1865.
 11. Cezar, M. F., and Sousa, W. H. 2007. Carcaças ovinas e caprinas - Obtenção, avaliação e classificação (1st. Ed). Uberaba: Editora Agropecuária Tropical, 232 p.
 12. Costa, S. B. M., Ferreira, M. A., Pessoa, R. A. S., Batista, A. M. V., Ramos, A. O., Conceição, M. G., and Gomes, L. H. S. 2012. Tifton hay, soybean hulls, and whole cottonseed as fiber source in spineless cactus diets for sheep. *Tropical Animal Health and Production*, 44, 1993–2000. DOI:10.1007/s11250-012-0169-2
 13. Duckett, S. K., Klein, T. A., and Leckie, R. K. 1998. Effect of freezing on calpastatina activity and tenderness of callipyge lamb. *Journal Animal Science*, 76, 1869–1874. DOI: 10.2527/1998.7671869x
 14. Gomes, J. C., and Oliveira, G. F. 2011. Análises físico-químicas de alimentos (1st ed.). Viçosa, MG: Ed. UFV.
 15. Hall, M. B. 2001 Calculation of non-structural carbohydrate content of feeds that contain non-protein nitrogen. Florida: University of Florida, p.A-25 (Bulletin,339).
 16. Huff-Lonergan, E. J., and Lonergan, S. M. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, 71, 194–204. DOI: 10.1016/j.meatsci.2005.04.022
 17. Hughes, J. M., Oiseth, S. K., Purslow, P. P., and Warner, R. D. 2014. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Science*, 98, 520–532. DOI: 10.1016/j.meatsci.2014.05.022
 18. Johnson, T. R., and Combs, D. K. 1991. Effects of prepartum diet, inert rumen bulk, and dietary polyethylene glycol on dry matter intake of lactating dairy cows. *Journal Dairy Science*, 74, 933–944. DOI: 10.3168/jds.S0022-0302(91)78243-X
 19. Knupp, L., Carvalho, F., Cannas, A., Marcondes, M., Silva, A., Francesconi, A., Beltrão da Cruz, G. R., Atzori, A. S., Gaspa, G., Costa, R. 2019. Meta-analysis of spineless cactus feeding to meat lambs: Performance and development of mathematical models to predict dry matter intake and average daily gain. *Animal*, 13, 2260–2267. doi:10.1017/S1751731119000326
 20. Martin, P., and Bateson, P. 2007. *Measuring behavior: an introductory guide* (3rd. ed.). New York: Cambridge: University Press.

21. Moura, M. S. C., Guim, A., Batista, A. M. V., Maciel, M. V., Cardoso, B. D., Lima Júnior, D. M., and Carvalho, F. F. R. 2020. The inclusion of spineless cactus in the diet of lambs increases fattening of the carcass. *Meat Science*, 160, 107975. <https://doi.org/10.1016/j.meatsci.2019.107975>
22. National Research Council - NRC. 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. Washington, D.C.: National Academy of Sciences, 2007.
23. Oliveira, A. P., Pereira, E. S., Biffani, S., Medeiros, A. N., Silva, A. M. A., Oliveira, R. L., and Marcondes, M. L. 2018. Meta-analysis of the energy and protein requirements of hair sheep raised in the tropical region of Brazil. *Journal of Animal Physiology and Animal Nutrition*, 102:e52–e60. doi: 10.1111/jpn.12700.
24. Pinho, R. M. A., Santos, E. M., Oliveira, J. S., Carvalho, G. G. P., Silva, T. C., Macedo, A. J. S., Corrêa, Y. R., and Zanine, A. M. 2018. Does the level of forage neutral detergent fiber affect the ruminal fermentation, digestibility and feeding behavior of goats fed cactus pear? *Animal Science Journal*, 89, 1424–1431. doi: 10.1111/asj.13043
25. Purchas, R. W., Davies, A. S., and Abdullah, A. Y. 1991. An objective measure of muscularity: changes with animal growth and differences between genetic lines of Southdown sheep. *Meat Science*, 30, 81–94. [https://doi.org/10.1016/0309-1740\(91\)90037-Q](https://doi.org/10.1016/0309-1740(91)90037-Q)
26. Ramos, E. M., and Gomide, L. A. M. 2009. Avaliação da qualidade de carnes: fundamentos e metodologias (1st ed). MG: Editora UFV.
27. Rocha Filho, R. R., Santos, D. C., Vêras, A. S. C., Siqueira, M. C., Novaes, B., Mora-luna, L. P., Monteiro, C. C. F., and Ferreira, M. A. 2021. Can spineless forage cactus be the queen of forage crops in dryland areas? *Journal of Arid Environments*, 186, 104426. <https://doi.org/10.1016/j.jaridenv.2020.104426>
28. Sáenz, C. P., Sepúlveda, E., and Matsuhiro, B. 2004. Opuntia spp mucilage's: a functional component with industrial perspectives. *Journal of Arid Environments*, 57, 275–290. [https://doi.org/10.1016/S0140-1963\(03\)00106-X](https://doi.org/10.1016/S0140-1963(03)00106-X)
29. Sano, H., Shibasaki, S., and Sawada, H. 2009. The effect of the source of nitrogen supplementation on nitrogen balance, rates of plasma leucine turnover, protein synthesis and degradation in sheep. *Journal Archives of Animal Nutrition*, 63, 401–412. <https://doi.org/10.1080/17450390903052698>
30. Santos, K. C., Carvalho, F. F. R., Carriero, M. M., Magalhães, A. L. R., Batista, A. M. V., Fagundes, G. M., and Bueno, I. C. S. 2020a. Use of different carbohydrate sources associated with urea and implications for in vitro fermentation and rumen microbial populations. *Animal Production Science*, 60, 1028–1038. DOI:10.1071/AN18633
31. Santos, K. C., Carvalho, F. F. R., Carriero, M. M., Magalhães, A. L. R., Silva, F. J. S., Cardoso, D. B., Souza, D. R., Silva, L. C. R. L., Nascimento, A. G. O., and Batista, A. M. V. 2020b. Can urea associated with alternative carbohydrate sources replace conventional concentrate for lambs?. *Livestock Science*, 239, 104172. <https://doi.org/10.1016/j.livsci.2020.104172>
32. Santos, R. D., Neves, A. L. A., Santos, D. C., Pereira, L. G. R., Gonçalves, L. C., Ferreira, A. L., Costa, C. T. F., Araujo, G. G. L., Scherer, C. B., and Sollenberger, L. E. 2018. Divergence in nutrient concentration, in vitro degradation and gas production potential of spineless cactus genotypes selected for insect

- resistance. *The Journal of Agricultural Science*, 156, 450-456. <https://doi.org/10.1017/S002185961800031X>
33. SAS. 2000. *Statistical Analyses System Institute, Inc 2000. SAS user's guide: Statics Version, 2000.* SAS, Cary, N. C.
34. Shen, J., Chen, Y., Moraes, L., Yu, Z., and Zhu, W. 2018. Effects of dietary protein sources and nisin on rumen fermentation, nutrient digestion, plasma metabolites, nitrogen utilization, and growth performance in growing lambs. *Journal of Animal Science*, 96, 1929–1938. [https://doi: 10.1093/jas/sky086](https://doi.org/10.1093/jas/sky086)
35. Sierra, I. 1986. La denominación de origen en el ternasco de Aragón. *Información Técnica Económica Agraria*, 66, 3–12.
36. Silva, F. J. S., Santos, K. C., Guim, A., Cabral, A. M. D., Maciel, M. V., Nascimento, A. G. O., Lima Júnior, D. M., and Carvalho, F. R. C. 2020. Body weight components and meat quality of hair sheep fed diets containing different carbohydrate sources associated with urea. *Chilean Journal of Agricultural Research*, 80, 361–369. doi:10.4067/S0718-58392020000300361
37. Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., and Russel, J. B. 1992. A net carbohydrate and protein system fort evaluating cattle diets. II carbohydrate and protein availability. *Journal of Dairy Science*, 70, 3562–3577. DOI: 10.2527/1992.70113562x
38. Pessoa, D. V., Pereira, A. A., Magalhães, A. L. R., Teodoro, A. L., Araújo G. G. L., Medeiros, A. N., Nascimento, D. B., and Cardoso, C. B. 2020. Forage nutritional differences within the genus *Opuntia*. *Journal of Arid Environments*, 181,1 04243. <https://doi.org/10.1016/j.jaridenv.2020.104243>
39. Valente, T. N. P., Detman E., Queiroz A. C., Valadares Filho, S. C., Gomes, D. I., and Figueiras, J. F. 2011. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. *Revista Brasileira de Zootecnia*, 40, 2565–2573. <https://doi.org/10.1590/S1516-35982011001100039>
40. Van Soest, P. J., Robertson, J. B., Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, v. 74, n. 10, p. 3583–3597.
41. Vieira, E.L., Batista, Â. M. V., Guim, A., Carvalho, F. F., Nascimento, A. C., Araújo, R. F. S., and Mustafa, A. F. 2008. Effects of hay inclusion on intake, *in vivo* nutrient utilization and ruminal fermentation of goats fed spineless cactus (*Opuntia fícus-indica* Mill) based diets. *Animal Feed Science and Technology*, 141, 199–208. <https://doi.org/10.1016/j.anifeedsci.2007.05.031>