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Effects of dietary inclusion of dry umbu fruit pulp residue (*Spondias tuberosa* Arr. Cam) on intake, ingestive behaviour, digestibility, nitrogen balance and ruminal pH in sheep

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ABSTRACT. The aim of the present study was to evaluate the effect of dietary inclusion of dry umbu pulp fruit residue (DUFR) into lamb diet. DUFR replaced ground corn at 0, 8, 16, 24 and 32% of the total diet. To evaluate intake, digestion, nitrogen (N) balance and ingestive behaviour, twenty-five uncastrated male lambs (40.8 ± 4.17 kg) were randomly distributed among treatments for 21 days. To evaluate ruminal pH, ammonia N in the rumen fluid and total protozoa abundance, five uncastrated Santa Ines male sheep (40.3 ± 3.70 kg) were used in a 5×5 Latin square design, for 75 days. The intake of dry matter (DM), crude protein (CP) and total digestible nutrients (TDN), CP and ether extract (EE) digestibilities, ruminal ammonia N, N-intake and N-balance were not linearly affected by the inclusion of DUFR. However, a linear increase in neutral detergent fibre (NDF_{ap}) intake, time spent eating and ruminating, the number of boluses chewed/day, and ruminal pH in sheep with the increase of DUFR inclusion were observed. The intake of EE and non-fibrous carbohydrates (NFC), digestibility of DM, NDF and TDN, time spent idling, amount (g) of DM/bolus chewed, and protozoa count decreased linearly with dietary inclusion of DUFR. The inclusion of up to 32% DUFR instead of ground corn stimulates eating and rumination time, however, it is not recommended as a concentrate feed because it reduces nutrient digestibility and nitrogen balance impairing animal performance.

Introduction

Fruit production is among sectors generating the most agroindustrial waste with potential use as animal feed at a low cost. Native perishable fruits, such as umbu (*Spondias tuberosa*, Anacardiaceae), are harvested only in the spring and summer, require immediate processing and consequently generate large volumes of waste that can be used as an alternative feed source for ruminants (Borges et al., 2007; Bezerra et al., 2015).

Umbu, also called Brazilian plum, is a native tree for tropical Brazilian regions and its fruits are commercialized '*in natura*' or as a frozen pulp and

considered a product of extractivism (Lima et al., 2002). The fruit is composed of a yellow-green peel and white-green, soft, juicy and pleasantly bitter-sweet flesh, rich in vitamin C and minerals and it is composed of 22% of peel, 68% of pulp and 10% of seeds, the residue of which is mostly composed of peel and seeds (Folegatti et al., 2003). Its fruits and leaves serve as fodder for small mammals as well as sheep and goats (Mertens et al., 2015).

During processing, a large amount of residue generated from husks and seeds is frequently discarded in the environment, as knowledge of exploitation techniques is often scarce. Two of the main fates of the residues generated during the production of umbu pulp are used as synthetic antioxidants (Lima et al., 2002) due to the presence of phenolic compounds as tannins. According to Lima et al. (2002) and Moreira et al. (2012), the tannin levels in umbu pulp ranged from 42 to 48 mg/100 g in fruit and reaching up 126.27 mg/100 g in the pulp. In ruminants, the tannin inclusion at high concentrations (>50 g/kg dry matter (DM)) in diet stimulates the formation of the protein-tannin complex in the rumen, reducing the microbial protein degradation (Mazza et al., 2020). Thus, Costa et al. (2008) in lambs fed tannin *ad libitum* observed a decrease in the availability of protein and polysaccharide due to bacteriostatic and bactericidal actions of tannin in the rumen, promoting degradation of the Gram-negative bacteria and inhibiting enzymatic activity (Orlandi et al., 2015), and consequently reducing intake and digestibility of feed (Hassanat and Benchaar, 2013; Al-Kindi et al., 2016), and lambs performance (García et al., 2017; Caldas et al., 2021) was observed.

However, included at moderate levels tannins can bring benefits: can form a tannin-protein complex in the ruminal environment, which limits the excessive degradation of the protein by ruminal microbiota; this complex is undone on low pH (such as the abomasum pH) causing a greater absorption of amino acids in the small intestine, increasing N-retained and N-basal endogenous and improving the use of dietary protein (Makkar, 2003; Burke et al., 2014; Min et al., 2015).

It was noted that in 2016 the umbu waste was around 8.4 t (IBGE, 2016). Such a waste has not been used for any other purpose and discarded in nature (Nascimento et al., 2016). The use of residue from tropical fruit (such as umbu), in addition to being inexpensive and an alternative product that is easily accessible in Brazil, can be used as a feed supply for animal needs and be a new source for farmers and producers to feed their herds – ensuring reduced costs and increased production (Folegatti et al., 2003; Mertens et al., 2015). Therefore, the level of dietary inclusion, associated with the action of bioactive compounds may enhance diet's protein utilization and microbial protein synthesis (Van Soest, 1994). This also affects the eating and ruminating behaviour and consequently animal performance. So, we hypothesised that the inclusion of up to 32% of dry umbu fruit residue (DUFR) into lamb diets would allow animals to maintain their performance even when the proportion of ground corn is reduced. Then, it is necessary to evaluate the optimum inclusion of this residue in the diet of sheep on intake, digestion, ingestive behaviour, N-balance and ruminal pH and the correlation among these variables.

Material and methods

Ethical considerations

The present study was conducted at the Federal University of Bahia, Salvador, Brazil, after approval by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (Protocol Number 18/2016), obeying the provisions of Law Nº 11,794 (October 8, 2008) and the standards published by the National Council of Control of Animal Experimentation (CONCEA).

Residue dehydration process

Dried umbu fruit residue (DUFR) used in the present study was donated by the fruit pulp agroindustry located in the municipality of Feira de Santana, Bahia, Brazil. The fruit pulp was dehydrated in the sun soon after it was delivered. Representative samples were collected for the determination of initial DM. Subsequently, the residue was spread onto plastic tarps in a single uniform layer of 4 cm on flat terrain and exposed to the sun from 8:00 to 18:00, before being covered by plastic tarpaulin during the night. The residue was stirred three times per day by hand to ensure homogeneous dehydration. During the dehydration process, the ambient temperature was evaluated with a digital thermohygrometer (Incoterm 7429 TFA, Porto Alegre, Brazil), the internal temperature of the residue was evaluated with an alcohol thermometer (Hongxing Instruments Factory, Hebei Province, China) at three different points, and 100 g of the material was collected to determine the DM at 1 h on the first day and every 2 h thereafter (Nascimento et al., 2020). The dehydration process was complete when the material reached a DM content higher than 80%, and the dried pulp was ground in a forage crusher.

Diets, treatments and management

All animals before the experiment were fed a similar diet, containing 16% of DUFR inclusion, to aloud the ruminal microbiota adaptation. The experimental diets were formulated based on the growth requirements of male sheep, with a target average daily gain (ADG) of 200 g as outlined by the NRC (2007). The food was administered twice a day, at 8:00 and 15:00, at the total mixture ration (TMR). The food included Tifton-85 hay (*Cynodon* sp.) ground into 5-cm pieces as roughage (40%) and a concentrated supplement (60%) composed of ground corn, soybean meal, a mineral premix and DUFR ground into approximately 3-mm pieces and added at a level of 0, 8, 16, 24 or 32% as DM of the total diet (Tables 1 and 2). The feed offered was adjusted daily and weighed to guarantee 10% refusal. Water was provided *ad libitum*.

Table 1. Chemical composition of ingredients used in the preparation of experimental diets

Chemical composition, g/kg dry matter	Dry umbu fruit residue	Ground corn	Soybean meal	Tifton-85 hay
Dry matter, g/kg as fed	904	885	910	890
Ash	34.1	14.7	64.1	67.1
Crude protein (CP)	46.5	71.4	507	51.2
Ether extract	17.4	36.9	6.50	7.70
Neutral detergent fibre$_{ap}$ [a]	596	103	107	768
Acid detergent fibre	386	75.2	52.1	305
Neutral detergent insoluble protein	282	218	105	329
Acid detergent insoluble protein, g/kg CP	135	110	60.5	68.7
Non-fibrous carbohydrates	305	774	293	106
Cellulose	354	22.2	59.8	259
Hemicellulose	211	68.1	53.9	434
Acid detergent lignin	31.6	12.6	13.4	45.4
Total digestible nutrients	686	914	880	569

[a] corrected for ash and protein

Experiment 1: Animals, intake, digestion, nitrogen balance, and ingestive behaviour

Twenty-five uncastrated Santa Ines male sheep with a mean age of six months and an initial body weight (BW) of 40.8 ± 4.17 kg were randomly distributed among treatments (5 treatments with 5 replicates). The animals were housed individually in metabolic cages and provided with drinking fountains, feeders and individual compartments for total faeces and urine collection separately. The experiment lasted 21 days, with 14 days of adaptation period and 7 days of leftover feed, faeces and urine sample collection. The nutrient intake was calculated by determining the difference between the total amount of each nutrient offered in the diet and the total amount of each nutrient contained in the leftover feed. The faeces and urine were quantified and collected using different buckets collector (one for faeces and the other for urine) in the metabolic cage. Then, the pH of the urine was checked, and an aliquot of 10% of the daily volume was collected. The samples were kept at a pH < 3 to avoid bacterial destruction of the metabolites present in the urine and then stored in plastic flasks at –20 °C for later use to quantify urinary concentrations of total nitrogen. A 10-ml aliquot of urine was added to a flask with 40 ml of 0.036 *N* sulfuric acid (H_2SO_4), and had the pH adjusted below 3 with sulphuric acid pure for analysis drops, and then was stored in a plastic flask at –10 °C for further analysis of uric acid and urea.

Table 2. Proportions of ingredients and chemical composition of experimental diets, dry matter (DM) basis

Variable	Umbu pulp fruit dry residue, % DM total				
	0	8	16	24	32
Ingredient proportion, g/kg DM					
ground corn	37.5	29.0	20.5	12.0	3.50
soybean meal	21.0	21.5	22.0	22.5	23.0
dry umbu fruit residue	0.0	8.0	16.0	24.0	32.0
mineral mixture[a]	1.50	1.50	1.50	1.50	1.50
Tifton-85 hay	40.0	40.0	40.0	40.0	40.0
Chemical composition, g/kg DM					
dry matter, g/kg as fed	894	895	896	896	897
ash	66.1	68.3	70.6	72.8	75.0
crude protein (CP)	151	151	150	150	150
ether extract	22.8	21.3	19.9	18.4	17.0
neutral detergent fibre$_{ap}$ [b]	373	411	450	488	527
neutral detergent insoluble protein g/kg CP	235	240	245	249	254
acid detergent insoluble protein, g/kg CP	81.4	83.2	84.9	86.7	88.4
cellulose	125	151	178	205	231
hemicellulose	211	222	233	245	256
acid detergent lignin	25.7	27.2	28.7	30.3	31.8
non-fibrous carbohydrates	387	348	310	271	232

[a] contained per kg of diet (as active elements): g: calcium 120, phosphorus 87, sodium 147, sulpfur 18; mg: copper 590, cobalt 40, chromium 20, iron 1 800, iodine 80, manganese 1 300, selenium 15, zinc 3 800, molybdenum 300, fluoride max. 870; [b] corrected for ash and protein

The digestibility coefficient was determined by the following equation:

$$CD = [(\text{amount (g) of nutrient intake} - \text{amount (g) of nutrient excretion in the faeces})]/(\text{amount (g) of nutrient intake}) \times 100.$$

Total digestible nutrient (TDN) intake was obtained from the difference between the amounts of each nutrient consumed and recovered in the faeces on a DM basis, according to the equation of Sniffen et al. (1992):

TDN intake (g/kg) = (digestible CP) + (2.25 × digestible ether extract (EE)) + (digestible non-fibrous carbohydrates (NFC)) + (digestible neutral detergent fibre (NDF)).

N-balance or retained (g/day) = N-intake – N-total excretion.

N-total excretion was calculated from the sum of the N-faecal and N-urinary excretion.

The NFC was determined using the equation reported by Mertens (1997):

NFC = 100 – ash – CP – EE – NDF.

The sheep were individually evaluated at five-minute intervals for 24 h (eating, ruminating and idling activities) by five trained observers (five animals per observer) on days 17, 19 and 21 to evaluate ingestive behaviour according to the method of Martin and Bateson (1993). The nighttime observations were conducted using artificial lighting. The observers counted both the number of ruminating chews and the number of cakes ruminated per day. Eating and ruminating efficiency rates expressed as g DM/h and g NDF/h were calculated by dividing the DM and NDF intake by the total time spent eating and/or ruminating within a 24-h period, according to Bürger et al. (2000). The total chewing time (TCT, h/day) was calculated as the sum of eating time and ruminating time.

Experiment 2: Animals, ruminal parameters, and experimental design

Five Santa Ines male sheep, approximately 12 months old, uncastrated, and with an approximate BW of 40.3 ± 3.70 kg, were cannulated in the rumen and individually housed in concrete bays in a 5×5 m space using a 5×5 Latin square design (5 treatments and 5 periods). These animals received the same diets on the same schedule as the animals in the metabolic cages. The experiment lasted for 75 days, and was divided into 5 periods with 15 days each. Each period was divided into 14 days of adaptation and 1 day of ruminal fluid collection. Ruminal fluid samples (250 ml) were collected immediately, directly in the rumen with the help of a sterilized container, before and 2, 4, and 6 h after feed delivery. To separate the liquid phase from the solid phase the ruminal content was initially filtrated in a nylon filter with a pore size of 100 µm for subsequent separation. The pH analyses were performed immediately after the collections using a pH meter. A part (50 ml) of the total ruminal fluid collected was mixed (1:1, v/v) with a 50% formalin solution to count protozoa according to the method described by D'Agosto and Carneiro (1999). The protozoa population count was obtained from an aliquot of rumen where the samples were diluted with 20% glycerol and stained by Lugol solution for counting. The concentration of ammonia nitrogen ($N\text{-}NH_3$) in the ruminal fluid was determined by colorimetry according to a method proposed by Foldager (1977). For deproteinization, 1 ml 10% sodium tungstate was added to the tubes containing the sample, and then the tubes were centrifuged at 3 000 rpm for 15 min. After that, 25 µl of the supernatants were pipetted and placed in test tubes. After that, 5 ml of phenol reagent and 5 ml of sodium hypochlorite reagent were added. The tubes were corked, stirred, and kept in a water bath at 37 °C for 15 min. With the aid of a spectrophotometer (Beijing Rayleigh AIC model VIS-7220, Beijing, China) set at 630 nm the absorbance readings were taken. The observed values were used to estimate ammonia nitrogen concentrations in mg/100 ml of ruminal liquid.

Chemical composition

Triplicate samples of ingredients, refusals, and faeces were predried at 55 °C for 72 h, ground with a Wiley mill (Tecnal TE-650, Piracicaba, Brazil), passed through a 1-mm sieve, stored in airtight plastic containers (ASS, Ribeirão Preto, Brazil), and properly sealed until analysed according to the Association of Official Analytical Chemists (AOAC International, 2012) recommendations concerning DM (method 930.15), N (method 968.06; urine was also evaluated), EE (method 920.29), and ash (method 942.05) (Tables 1 and 2). The analyses of NDF and ADF were performed according to Van Soest et al. (1991), with modifications to implement the use of an autoclave as proposed by Senger et al. (2008). The autoclave temperature was maintained at 110 °C for 40 min. The NDF residue was incinerated in an oven at 600 °C for 4 h, and the protein correction was determined by subtracting the neutral detergent-insoluble nitrogen (NDIN). The NDIN and acid detergent-insoluble nitrogen (ADIN) contents were determined according to the methods of Licitra et al. (1996). Acid detergent lignin (ADL) was determined according to method 973.18 (AOAC International, 2002), and the ADF residue was treated with 72% sulphuric acid. NFC was determined by the equation of Mertens (1997).

Statistical analyses

The values obtained were tested for normality of residuals by using the Shapiro-Wilk test in the ExpDes package in R i386 3.3.1®. When the normality assumption was met, the data were submit-

ted to analysis of variance (ANOVA) and regression analysis (with 5% significance) with the ExpDes package in R i386 3.3.1®.

The statistical analyses of intake, digestion, N balance and ingestive behaviour were conducted following a completely randomized design with five levels of DUFR (0, 8, 16, 24 and 32% total DM) and five replicates, according to the model:

$$Yij = \mu + Si + eij,$$

where: Yij – observed value, μ – general mean, Si – effect of residue inclusion level, and eij – effect of experimental error in the plots.

For pH, protozoan abundance, and the concentration of ammonia nitrogen in rumen fluid, the analyses were conducted following a Latin square design according to the model:

$$Yijk = M + Li + Cj + Tk (ij) + eijk,$$

where: Yijk – value observed in the experimental unit that received treatment k (in row i and column j), M – effect of the general mean, Li – effect of line i (animal), Cj – effect of column j (period), Tk (ij) – treatment effect k applied to line i and column j (inclusion levels of the residue, 0, 8, 16, 24 and 32% DM), and eijk – random error (residual).

Ducan post-hoc mean analysis between groups was added to compare means. The effects were considered significant at $P < 0.05$ and the means were presented as mean per treatment followed by the standard error of the mean (SEM) per studied variable.

Results

Residue dehydration process

In total, 41 h of exposure to the sun and local climatic conditions distributed over 4 days was necessary to dehydrate the residue from DUFR extraction. At the beginning of the dehydration process, the residue consisted of 15% DM, and at the end – 80.8% DM. The surface temperature of the residue was the highest (41.7 °C) at 14:00 on the fourth day of processing, and the lowest (22.6 °C) at 18:00 on the second day of processing, with a mean of 31.5 °C. The internal temperature of the residue was the highest (34 °C) at 14:00 on the second day of processing, and the lowest (25 °C) at 18:00 on the same day, with a mean of 29.4 °C.

Experiment 1: Nutrient intake, ingestive behavior, digestion and nitrogen (N) balance

The replacement of ground corn with dehydrated DUFR in the sheep diet did not affect ($P > 0.05$) the intake of DM (g/day and %BW), CP (g/day) and TDN (g/day; Table 3) in the regression analysis (linear and quadratic) and analysis of variance (mean comparison test).

There was a linear increase in NDF_{ap} intake (in g/day and %BW) and the proportional intake of NDF_{ap} with the dietary inclusion of DUFR. The inclusion of DUFR into the diet promoted a linear decrease in the intake (g/day) and proportional intake of EE and NFC.

The mean comparison test showed that the inclusion of levels above 24% of DUFR (24 and 32%) caused an increase in the intake and in the proportional intake of NDF_{ap} in comparison to the control treatment (0%). On the other hand, the intake (g/day) and proportional intake (g/day) of EE and NFC were lower in sheep fed diet containing 32% DUFR in comparison to treatments with 0 and 8% DUFR.

There was a linear reduction in the digestibility of DM, NDF_{ap}, NFC and TDN with dietary inclusion of DUFR. The digestibility of CP and EE were not linearly influenced by the inclusion of up to 32% DUFR into diet.

The mean comparison test indicated that the NDF_{ap} digestibility was similar for inclusion levels of 0, 8 and 16%, which showed higher digestibility than in groups with 24 and 32% DUFR. On the other hand, this behaviour was in contrast to the NFC digestibility – inclusion of 0, 8 and 16% resulted in lower digestibility than treatments with 24 and 32% DUFR. In the control group the highest digestibility of TDN in comparison to other groups was observed.

The inclusion of DUFR promoted a linear increase in N-urinary and N-total excretions (g/day and %N-intake) and a linear reduction in N-retained (g/day and %N-intake). There was no linear effect of DUFR inclusion in the N-intake, and N-faecal excretion (g/day and %N-intake).

Applying the mean comparison test, lower urinary-N and total-N excretions (g/day and %N-intake) were observed in sheep fed diets with 0 and 8% DURF in comparison to sheep fed with 24 and 32% DUFR addition. On the contrary, an increase in N-retained in animals fed diets with 0 and 8% DUFR was observed when compared to animals fed diets with 24 and 32% DUFR inclusion

The time spent eating and ruminating linearly increased with the replacement of ground corn with DUFR in the diet (Table 4). On other hand, the time spent idling presented a linear decrease along with the addition of DUFR. The eating rate of DM (g/h) was linearly reduced with the inclusion of DURF,

Table 3. Feed intake, digestibility and nitrogen balance of sheep fed dry umbu fruit residue (DUFR)

Item	DUFR, % DM					SEM	*P*-value	
	0	8	16	24	32		L	Q
Intake, g/day								
dry matter (DM)	1504	1436	1360	1450	1300	50.1	0.722	0.854
crude protein (CP)	244	231	224	241	201	9.10	0.623	0.654
neutral detergent fibre (NDF)*	388^{b}	541^{ab}	552^{ab}	641^{a}	647^{a}	30.9	0.004	0.346
ether extract (EE)	38.9^{a}	36.9^{a}	30.9^{ab}	31.2^{ab}	26.3^{b}	1.40	0.002	0.915
non-fibrous carbohydrates (NFC)	626^{a}	560^{ab}	454^{bc}	427^{c}	295^{d}	27.5	<0.001	0.717
total digestible nutrients (TDN)	1159	1110	967	971	943	50.8	0.125	0.600
Intake, % body weight (BW)								
DM	3.56	3.37	3.47	3.37	3.21	0.09	0.544	0.853
NDF*	1.18^{c}	1.27^{bc}	1.41^{ab}	1.56^{a}	1.59^{a}	0.04	<0.001	0.679
Proportional intake diet, g/day								
DM, as fed	93.4	93.1	92.7	92.3	92.1	0.11	0.388	0.966
CP	16.6	16.1	16.4	16.6	15.4	0.18	0.143	0.244
NDF*	31.6^{e}	37.7^{d}	40.6^{c}	44.1^{b}	49.8^{a}	1.28	<0.001	0.777
EE	2.70^{a}	2.45^{b}	2.27^{c}	2.15^{cd}	2.04^{d}	0.05	<0.001	0.069
NFC	42.5^{a}	37.0^{b}	33.4^{c}	29.5^{d}	24.8^{e}	1.26	<0.001	0.380
Digestibility, g/kg DM intake								
DM	787	735	733	707	683	2.06	0.001	0.678
CP	799	768	777	745	756	1.15	0.875	0.628
NDF*	726^{a}	685^{a}	683^{a}	669^{b}	668^{b}	2.06	0.001	0.862
EE	708	723	712	709	711	1.83	0.904	0.895
NFC	839^{a}	828^{a}	795^{ab}	757^{bc}	750^{c}	2.5	<0.001	0.607
TDN	685^{a}	620^{b}	548^{c}	548^{c}	525^{c}	1.53	<0.001	0.063
Nitrogen (N) balance, g/day								
N-intake	31.8	36.9	35.8	38.5	32.1	1.58	0.852	0.175
N-faecal excretion	7.43	8.58	7.87	9.34	7.45	0.32	0.713	0.145
N-urinary excretion	13.9^{c}	15.5^{c}	18.3^{b}	21.2^{ab}	22.1a	0.72	0.006	0.070
N-total excretion	21.3^{c}	24.1^{cb}	26.2^{b}	30.5^{a}	29.6^{a}	0.99	0.023	0.063
N-balance/retained	10.5^{ab}	12.82^{a}	9.63^{b}	7.96^{b}	2.55^{c}	1.09	0.005	0.284
N-excretion as %N-intake								
N-faecal	23.4	23.3	22.0	24.3	23.2	2.12	0.735	0.272
N-urinary	43.7^{b}	42.0^{b}	51.1^{ab}	55.1^{ab}	68.8^{a}	3.27	0.002	0.172
N-total	67.1^{c}	65.3^{c}	73.1^{bc}	79.3^{b}	92.1^{a}	3.99	<0.001	0.113
N-balance/retained	32.9^{a}	34.7^{a}	26.9^{b}	20.7^{c}	7.94^{d}	2.76	<0.001	0.128

SEM – standard error of the mean; L – linear, Q – quadratic; *correceted for ash and protein; $^{a–e}$ means with different superscripts within the row are significantly different at $P \leq 0.05$ (according to Duncan test)

while the eating rate of NDF and ruminating rate increced linearly. The mean comparison test showed that no differences were found for time spent (min/day) and eating rate (g/h) between levels of 16, 24 and 32% DUFR inclusion.

There was a linear decrease in the amount (g of DM/bolus) chewed and a linear increase in the number of boluses chewed per day when DUFR was used as a cor replacement.

The mean comparison test showed that the treatment without DUFR inclusion (0%) presented the lowest number of boluses chewed per day and NDF ruminating rate and higher (g DM/bolus) chewed in comparison to the other treatments.

Experiment 2: Ruminal parameters

The inclusion of DUFR as a replacement for ground corn linearly increased the mean ruminal pH and quantitatively reduced the protozoan abundance in the ruminal fluid of the sheep (Table 5). There was no interaction between ruminal fluid sampling time for pH/h analysis and DUFR. Nevertheless, the inclusion of the DUFR did not quantitatively affect the concentration of ammoniacal nitrogen.

The mean comparison test demonstrated that the treatment without DUFR inclusion (0%) presented lower pH and higher protozoan count when compared to treatments with inclusion above 16% of DURF (Table 5).

Table 4. Ingestive behaviour of sheep fed diets with dry umbu fruit residue (DUFR)

Variables	DUFR, % dry matter (DM) total					SEM	*P*-value	
	0	8	16	24	32		L	Q
Time spent, min/day								
eating	191^{bc}	177^{c}	212^{abc}	258^{a}	236^{ab}	9.42	0.007	0.716
ruminating	366^{b}	448^{ab}	512^{a}	466^{ab}	464^{ab}	16.66	0.026	0.003
idling	883^{a}	815^{ab}	716^{b}	716^{b}	740^{b}	21.06	0.006	0.072
Eating rate, g/h								
DM	403^{ab}	453^{a}	344^{b}	306^{b}	315^{b}	22.50	0.005	0.928
NDF	111^{b}	160^{a}	141^{ab}	165^{a}	179^{a}	8.78	0.005	0.702
Ruminating rate, g/h								
DM	196	193	159	185	173	6.65	0.270	0.478
NDF	57.8^{c}	71.3^{bc}	69.4^{bc}	82.8^{b}	103^{a}	3.43	<0.001	0.273
Chewing								
g DM/bolus	2.77^{a}	2.38^{b}	2.41^{b}	2.16^{c}	2.11^{c}	0.10	<0.001	0.084
Nº bolus/day	429^{b}	613^{a}	571^{a}	669^{a}	624^{a}	23.7	0.003	0.532

SEM – standard error of the mean; L – linear, Q – quadratic; NDF – neutral detergent fibre; ^{abc} means with different superscripts within the row are significantly different at $P \leq 0.05$ (according to Duncan test)

Table 5. Ruminal pH, ammonia nitrogen and protozoan population of sheep fed dry umbu fruit residue (DUFR)

Item	DUFR, % DM total					SEM	*P*-value	
	0	8	16	24	32		L	Q
pH mean	6.25^{c}	6.30^{bc}	6.48^{ab}	6.59^{a}	6.61^{a}	0.035	0.017	0.756
pH/h*								
0 h	6.46	6.47	6.75	6.87	6.77	0.079	0.018	0.390
2 h	6.15	6.33	6.43	6.57	6.58	0.060	0.005	0.448
4 h	6.07	6.20	6.41	6.47	6.54	0.064	0.005	0.518
6 h	6.33	6.19	6.33	6.44	6.56	0.062	0.039	0.225
N-NH_3 mean	9.87	10.27	10.25	10.79	9.98	0.332	0.946	0.650
N-NH_3/h**								
0 h	8.77	9.15	10.5	10.1	6,80	0,309	0.506	0.301
2 h	11.0	12.3	11.75	10.5	9.74	0.311	0.414	0.625
4 h	10.9	11.8	9.39	10.7	11.7	0.331	0.964	0.570
6 h	8.85	7.89	9.31	11.8	11.7	0.361	0.140	0.759
Protozoan***	5.59^{a}	5.17^{b}	5.05^{b}	3.17^{c}	2.08^{d}	0.407	0.005	0.068

SEM – standard error of the mean; L – linear, Q – quadratic; * there was no interaction between collection time and levels tested ($P = 0.8273$); ** concentration of ammonia nitrogen in rumen fluid (mg/dl); *** protozoan count $\times 10^5$ per ml of ruminal fluid; $^{a–d}$ means with different superscripts within the row are significantly different at $P \leq 0.05$ (according to Duncan test)

Discussion

The umbu pulp residue drying process in the sun was efficient in dehydrating the material, allowing to reach a DM content higher than 80% and preventing it from fermenting, and also enables material conservation for further use as animal feed in the dry season of the year (Nascimento et al., 2020). The local climatic conditions and the stirring of the residue during the process prevented deleterious microorganisms from fermenting the residue and raising the internal temperature of the material (Nascimento et al., 2020). This efficiency was verified by the maintenance of the internal temperature within 2 °C of the ambient temperature (Jobim et al., 2007).

The surface temperature of the DUFR at most of the analyzed points was higher than the internal temperature, which can be explained by the amount of solar radiation received by the upper layer. The difference between the superficial and internal temperatures was smaller in the initial and final hours of the day due to the lower solar incidence. The only time when the internal temperature of the residue was at least 2 °C different from the ambient temperature was at 14:00 on the first day of drying, when the ambient temperature was 30.3 °C, the internal temperature was 34 °C and the superficial temperature was 36.7 °C. The internal or superficial temperature did not surpass 40 °C at any time during the processing of the residue, which could accelerate the Maillard reaction.

The DM content of the diet was similar, even with DUFR inclusion replacing ground corn. Proportional diet intake was influenced by dietary inclusion of DUFR. It was shown that there was no preferential selection of the roughage over the concentrate, as shown by the similar proportions of effective CP intake between treatments. The amount of nutrients proportionally consumed in comparison to the proportion of nutrients offered can indicate whether animals preferentially consumed the concentrate or the roughage (Folegatti et al., 2003; Mertens et al., 2015). There was no preferential selection of the concentrate over the roughage, as shown by the equality between the proportion of CP proportionally consumed and the DMI. Therefore, the intake of NDF, EE and NFC in g/day and the proportional intake increased with the availability of these nutrients in the offered diet (Mazza et al., 2020).

The DUFR has 80% more NDF_{ap} in comparison to ground corn and 60% less NFC, which consequently increased NDF_{ap} and reduced NFC intake. Thus, despite the inclusion of DUFR did not influence the DMI, the greater fibre concentration in DUFR increased the time spent eating and ruminating and reduced the time spent idling. Consequently, there was an increase in chewing (Nº bolus/day) and eating and ruminating efficiencies of the DM, also sheep reduced eating efficiencies of DM and g DM/bolus chewed. In addition, DUFR contained more cellulose (354 g/kg in DM) than hemicellulose (211 g/kg DM), and, among the other concentrate ingredients, presented the highest value for ADL (31.6 g/kg as DM basis), factors that negatively affect fibre digestibility (Van Soest et al., 1991; Sniffen et al., 1992; Galvão et al., 2020). According to Van Soest (1994), the greater the representation of roughage ingredients in the diet, the longer the ruminating time. However, in the present study, the inclusion of up to 19.6% DUFR increased the time spent ruminating; the higher levels of DUFR inclusion had less pronounced effect.

In general, fruit residues as DUFR, show a high variation in their NDF_{ap} content, due to differences in cell wall composition (Azevêdo et al., 2011). The proportion of each cell wall component influences the fibre intake, mainly because they affect its digestibility that in turn affects nutrient intake (Van Soest et al., 1991). Another factor related to the greater Nº bolus chewed/day and lower eating efficiency of DM, TDN and NFC. The lower digestibility of NFC with DUFR inclusion into sheep diet in comparison to the control diet could be explained by the greater lignin content of these residues as also observed by Nascimento et al. (2016). The presence of lignin tends to increase the indigestible fraction of the fibre, reducing the potentially digestible fraction which can influence the cellulolytic enzymes and microbial metabolism (Abdullah et al., 2018).

In addition, DUFR can promote an increase in tannins content of the diet, which can negatively affect fibrolytic bacteria by changing its membrane, thus reducing enzymatic activity and its ability to bind to the tannin enzymes, decreasing its activity and thus causing lower fibre degradability (Makkar, 2003; Caldas et al., 2021). Moreira et al. (2012) observed tannin levels in umbu fruit ranging from 7.0 to 12.5%. Some researchers have reported that the presence of a high tannin concentration (>4% DM) in fruit residues results in the inhibition of voluntary intake and increases the number of chews due to astringents resulting from the formation of complexes by salivary proteins and tannin (Muir, 2011; Bezerra et al., 2015; Abdullah et al., 2018; Souza et al., 2018). High tannin intake (>50 g/kg DM) exerts bacteriostatic and bactericidal effects, which are due to the reduced availability of protein and polysaccharide (Costa et al., 2008), destruction of cell membranes of Gram-negative bacteria (Bhatta et al., 2009) or enzymes inhibition (Orlandi et al., 2015).

The inclusion of the tannins from DUFR may bring benefits by forming a tannin-protein complex in the ruminal environment, which limits the excessive degradation of the protein in the rumen, promoting a CP escape from the rumen (Makkar 2003; Ngwa et al., 2000; Abdullah et al., 2018). However, the amount of tannin available in the diet might impact the bond tannin-protein occurrence and its effect on N digestibility (Ngwa et al., 2000). In DUFR there is a low tannin concentration (0.40 mg; Costa et al., 2015) and a high concentration of acid detergent lignin as DM (7.79%; Azevêdo et al., 2011). Thus, the bond between protein and tannin will not occur effectively, which will impact the N metabolization and N-excretion, throughout faeces and urine (Mousa, 2011).

The ruminal pH observed in sheep fed diets including DUFR was within the normal range, between 5.5 and 7.4, and varied throughout the day according to feed administered and the time interval of the last feeding (Mazza et al., 2020).

The DUFR has a potential to replace roughage in diets for ruminants. In our study, we challenged the animals by replacing the ground corn in the concentrated supplement with DUFR, which resulted in the increased NDF content in the diet and probably pronouncing rumen filling effect, which depending

on the DUFR level, may restrict DMI. Further studies using this residue as a source of fibre, and observing effects in animal performance and meat quality are needed. In addition, the use of DUFR as a ground corn replacer will reduce the cost of the diet and also allow for using more natural resource as a feed.

Conclusions

The inclusion of up to 32% dried umbu fruit residue (DUFR) replacing ground corn in the supplemental concentrate increased fibre content and stimulates eating and ruminating behaviour in sheep. It also positively affected the ruminal pH. However, the use of DUFR is not recommended as a concentrated feed/ingredient, because it reduces the digestibility of nutrients and N-balance, and so impair animal performance.

Conflict of interest

The authors declare that there is no conflict of interest.

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