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Rumen Degradation of Endosperm and Mesocarp Expellers from *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. in Sheep Grazing Either Natural Pastures or *Brachiaria brizantha* cv. Marandu

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Simple Summary: Sheep producers in many countries of the world usually feed their animals with natural or improved pastures. In these situations, pastures alone usually do not cover animal needs, and supplementation is required. By-products from the oil industry are extensively used in animal feeding, and endosperm and mesocarp expellers from grugru palm may play an important role in animal feeding in the countries where it is distributed. To assess the protein value of an ingredient, the extent and rate of rumen degradation are central characteristics. However, associative effects with other components of the diet occur, and rumen degradation of individual ingredients should be assessed in the usual feeding conditions of an animal. On these grounds, the objective of this study was to provide information about rumen degradation of endosperm and mesocarp expellers from grugru palm in sheep grazing natural or cultivated monophytic pastures, and supplemented with a mixture of both expellers. The use of this mixture slows down the rate of degradation of the pastures, the effect of which is more intense in animals grazing natural swards. Supplementation with this mixture also increases the average daily gain of sheep, more substantially with multi-species natural pastures.

Abstract: Twenty-four ewes (eight fistulated in the rumen) were assigned to a 2 × 2 factorial design. The treatments included the pasture grazed (natural mixed swards—NMS or cultivated monophytic (*Brachiaria brizantha* cv. Marandu) swards—CMS) and the level of supplementation: without supplement or with a 1% supplement (dry matter (DM)/live weight). The supplement included one-third endosperm expeller (ENE) and two-thirds mesocarp expeller (ME) from *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. In sacco rumen degradation of ENE, ME, NMS, and CMS was assessed in fistulated sheep, as were rumen fermentation variables. Average daily gain (ADG) was assessed in non-fistulated animals during a 7-week period. Potential degradability of the DM and crude protein of the expellers was not affected by the type of pasture grazed or the level of supplementation ($p > 0.05$), but the fractional rate of degradation (c) of DM was three times faster ($p < 0.01$) for ME than for ENE. The potential degradability of neutral detergent fiber was 34% higher ($p < 0.0001$) for ENE, with no differences in c ($p > 0.1$). Supplementation slowed down the c of the DM of the pastures, especially in animals grazing NMS (24% lower). Treatments affected rumen pH, concentration of volatile fatty acids, and proportion of valerate, in different ways. The use of the supplement increased ADG of sheep (six-fold in sheep grazing NMS and 40% in those grazing CMS). The use of a mixture of one-third ENE plus two-thirds ME as a protein supplement in sheep grazing either multi-species natural pastures or monophytic swards of *Brachiaria brizantha* cv. Marandu slows down the fractional rate of degradation of the pastures, the effect of which is more intense in animals grazing natural swards. This supplementation also increases average daily gain, more substantially with multi-species natural pastures.

Keywords: ruminants; extensive systems; oil industry; by-products; rumen function

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1. Introduction

Grugru palm (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.) is widely distributed throughout the American continents, especially in Latin America, and has the potential to be introduced in Central Africa, Southern Asia, and Northern Australia as a bioenergetic source [1]. Although the predominant use of this species is in the production of bioenergy [2], it has been also reported for pharmaceuticals [3], medicine [4], human food [5], animal feed [6], and even in the masonry industry [7]. Oil extraction generates by-products such as endosperm (ENE) or mesocarp (ME) expellers, which can be efficiently used in animal feeding [6,8,9]. It is estimated that 20 t of fruits are produced per ha and year, from which ca. 0.9 t of ENE and 5.2 t of ME are obtained [10]. The processing of grugru palm fruits to obtain ENE and ME has been described by Loup [11]. Roughly, the fruits are stored for up to several months in containers to help keep them dry. During this period, some mechanical separation of the pericarp and the mesocarp occurs. After the storing–drying period, the pericarp is mechanically separated from the rest of the fruit, and then the mesocarp is pressed to obtain the mesocarp oil. The by-product of the oil extraction is known as ME. On its side, the endocarp is broken down and the endosperm is chemically separated with the aid of dihydrate kaolinite, which creates a density gradient. This way, the endosperm can be collected at the top of the tank after washing to eliminate the chemical. Then, it is dried down to a maximum of 9% dry matter, and ground and pressed to obtain the endosperm oil. The by-product of the oil extraction is known as ENE.

The published literature dealing with the chemical composition of both ENE and ME is very scarce [12], but data (n = 45 for ENE, and n = 25 for ME) from the Laboratory of Bromatology, Nutrition, and Animal Feeding of the Faculty of Veterinary Sciences, of the National University of Asunción, in Paraguay, indicate that ENE is a protein-rich by-product (346 ± 15.2 g crude protein/kg dry matter) with a moderate content in ether extract (98.7 ± 27.90 g/kg dry matter). On the other hand, ME is a low-protein (63.6 g/kg dry matter) by-product with a higher content in ether extract (146 ± 16.4 g/kg dry matter) than ENE. As a result, ENE could be used as a protein supplement and ME as an energy supplement. However, and due to the lack of published information on the digestion and performance of the animals consuming them, the criterion for its use by the producers is just their chemical composition.

The extent of protein degradation in the rumen is considered central to systems that have been proposed for the evaluation of protein requirements for ruminants [13]. These systems consider the microbial need for rumen-degradable N and also the host animal's need for amino acids derived either from microbial protein or from undegraded protein from the feed. The extent of degradation determines both the degradable part available for the rumen microbes and the undegraded protein that may be available for host animal enzymic digestion. Not only the extent of degradation but also the rate of degradation is extremely important [13].

When assessing rumen degradation of individual ingredients of a ration, it must be considered that it can be influenced by associative effects with the other components of the diet (e.g., forage vs. cereal grains [14]). Assessment of rumen degradation of any feedstuff should then be carried out in the usual feeding conditions of a determined animal. In this respect, sheep producers in the countries where grugru palm is more broadly distributed usually feed their animals with natural [15] or improved [16] pastures. Moreover, when by-products of *Acrocomia aculeta* (Jacq.) Lodd. ex Mart. are used, they are fed in the form of cake, a mixture of ENE and ME [6,8].

The main objective of the present paper was to provide information about rumen degradation of ENE and ME from *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. in sheep grazing either natural pastures or cultivated *Brachiaria brizantha* cv. Marandu. The influence of supplementing the sheep with a mixture of ENE and ME on rumen degradation of ENE and ME was also studied. The hypothesis is that the diet consumed by grazing sheep (type of pasture grazed, and supplementation or not with a mixture of one-third ENE and two-thirds ME) will affect rumen degradation of ENE and ME, rumen fermentation, and animal performance.

2. Materials and Methods

The trial was carried out at the sheep farm Condominio Stanley, in the Department of Caaguazú, San José de los Arroyos district, 122 km from Asunción, capital city of Paraguay (25°40′31.29″ S, 56°41′19.92″ W).

2.1. Experimental Design

Twenty-four non-pregnant, non-lactating Hampshire Down cross-bred grazing ewes (36 ± 3.8 kg (SEM) at the beginning of the experiment) were randomly assigned to four treatments (six ewes/treatment) in a 2 × 2 factorial arrangement. The factors were the type of pasture grazed (natural mixed swards (NMS) or cultivated monophytic swards (CMS) of *Brachiaria brizantha* cv. Marandu), and the level of supplementation (non-supplemented (NS) or supplemented (S) with a mixture of one-third ENE and two-thirds ME at a daily rate of 1% live weight (LW) on a dry matter basis). Weight homogeneity of the four animal groups was sought, and two animals assigned to each treatment were fistulated in the rumen 30 days before the adaptation period to the experimental diets. Four paddocks of 15,000 m^2 each were fenced, two of them with NMS and the other two with CMS. Within each type of pasture, one of the paddocks was supplemented, whereas the other one was not. Animals had access to automatic drinkers (one per paddock) during the whole experiment. The most important species of NMS were *Andropogon lateralis* Nees and *Axonopus compressus* (Sw.) P.Beauv. (*ca*. 14% cover each), *Sorghastrum agrostoides* (Speg.) Hitchc. (*ca*. 8% cover), *Paspalum notatum* Flüggé (*ca*. 7% cover), *Elyonurus latiflorus* (Nees ex Steud.) Hack and *Imperata brasiliensis* Trin. (*ca*. 3% cover each), *Paspalum devincenzii* Parodi (*ca*. 2% cover), *Paspalum guaraniticum* Parodi var. crovettoi and *Axonopus iridaceus* (Mez) Hitchc. & Chase ex Rojas (*ca*. 1.7% cover each), and *Paspalum maculosum* Trin. (*ca*. 1.3% cover). Other species present in the sward, even though at low percentages of cover, were *Mimosa* spp. and *Vernonia chamaedrys* Less. The supplement was offered at 17:00 in troughs installed in each paddock (one per paddock, shared by all animals in a paddock). Animals had access to the assigned pasture for 12 h each day (from 7:30 am till 7:30 pm), and then were housed in wooden barns, made exclusively for the trial, during the night. All the supplement provided was consumed by the animals, but no measurement of pasture intake could be made. In this respect, exclusion cages were not available at the farm to estimate pasture intake from agronomic measurements. Apart from this unavailability, this technique does not take into account the selection exerted by the animals on different species and plant parts, and this could be important in sheep grazing NMS. The *n*-alkanes technique was discarded due to its numerous odds (need for fecal recovery of the odd-chain alkanes present in the biomass consumed, for example). Calculation of pasture intake by subtracting the concentrate contribution from animal requirements was considered inaccurate. Weekly samples of pastures from the four paddocks were collected to assess the evolution of chemical composition. Both ME and ENE were obtained as described by Loup [10] and provided by Industrial Aceitera SACI (Capiatá, Paraguay). Samples of the two swards, the two expellers, and the supplement, were ground in a hammer mill fitted with a 1 mm sieve size, and analyzed at the Laboratory of Bromatology, Nutrition, and Animal Feeding, Faculty of Veterinary Sciences, National University of Asunción, Paraguay. The procedures of AOAC [17] were followed for dry matter (DM; ref. 934.01), organic matter (OM; ref. 942.05), crude protein (CP; ref. 976.05), and ether extract (EE; ref. 2003.05). Concentration of neutral detergent fiber (aNDFom) was analyzed as described [18], using α-amylase and sodium sulfite, and results were expressed exclusive of residual ashes. Acid detergent fiber (ADFom) and acid detergent lignin (Lignin (sa)) were analyzed as described by AOAC [17] (ref. 973.18), and Robertson and Van Soest [19], respectively. Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were obtained as in [20]. The chemical composition of pastures, expellers, and supplement is given in Table 1.

Table 1. Nutrient composition (g/kg dry matter ± standard error of the mean) of natural mixed swards (NMS), cultivated monophytic swards (CMS) of *Brachiaria brizantha* cv. Marandu, mesocarp (ME), and endosperm (ENE) expellers of *Acrocomia aculeata* (Jacq.) Lodd. ex Mart., and supplement made of 1/3 ENE and 2/3 ME (n = 32 for NMS and CMS).

	NMS	**CMS**	**ME**	**ENE**	**Supplement**
Dry matter (g/kg)	n.d.	n.d.	914	940	925
OM	843 ± 17.3	834 ± 20.0	840	852	826
CP	51 ± 7.7	47 ± 7.9	83	344	226
NDF	711 ± 22.1	652 ± 24.1	586	305	422
NDIN	n.d.	n.d.	61	333	144
ADF	372 ± 17.0	304 ± 10.4	335	260	252
ADIN	26 ± 3.5	20 ± 4.8	26	93	66
ADL	55 ± 3.0	31 ± 4.5	153	103	105
EE	30 ± 5.6	31 ± 4.5	175	150	166
ME (Mjul/kg dry matter)	7.99	9.62	8.87	10.68.	10.87

OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; NIDN, neutral detergent insoluble nitrogen, ADF, acid detergent fiber; ADIN, acid detergent insoluble nitrogen; ADL, acid detergent lignin; EE, ether extract. ME, metabolizable energy estimated from ADF content according to the equation proposed by Mertens [21] (net energy for lactation (NE; kcal/kg dry matter) = (2.469–0.0351) · % acid detergent fiber; R^2 = 0.849; Metabolizable Energy = NE/0.61); n.d., not determined.

The care and management of animals were performed according to the Spanish Policy for Animal Protection RD 1201/05, which meets the EU Directive 86/609 on the protection of animals used for experimental and other scientific purposes, and the experimental protocol was approved by the Ethical Committee for Animal Research of the University of Zaragoza (PI48/20). The protocols applied were also covered by the Protection and Animal Welfare Act 4840/13, from the Government of Paraguay.

Non-fistulated sheep (four per treatment) were exclusively used for productive performance studies (average daily gain-ADG) during a 7-week period during the time of the year with more pasture productivity and quality. The ADG of fistulated animals was not included in order to avoid any influence of the surgery on that variable. Once a week, at 7:30 h, all ewes were weighed (with a precision of ±100 g) before taking them to the pasture, and ADG was estimated as the regression coefficient of individual LW on time.

2.2. Rumen Degradation of Pastures and Expellers

After the diet adaptation period, which lasted 12 days, NMS, CMS, ME, and ENE were incubated in polyester bags (10 × 16 cm; 48 µm pore size), in the rumen of the fistulated ewes, for 2, 4, 8, 16, 24, 48, and 72 h after first accession to the pastures. The pastures were incubated in the animals grazing them (n = 4,) whereas the expellers were incubated in all fistulated animals (n = 8). Rumen degradability of DM, crude protein (CP), and neutral detergent fiber (NDF) was assessed following the procedures described by Vanzant et al. [22]. About 5 g of the ground (2 mm) feedstuffs was incubated in duplicate bags per incubation time and ewe. Samples of pastures for incubation were taken from five 1 m^2 squares thrown randomly within each type of sward. The content inside each square was manually reaped at a height of 2–3 cm from the ground for NMS, and at 10 cm from the ground for CMS. The content of the five squares within each sward was pooled and dried in a forced-air oven at 60 °C for 48 h. Bags withdrawn from the rumen after each incubation time were rinsed with cool (*ca.* 15 °C) tap water and then frozen at −20 °C. Once all bags had been withdrawn, they were washed for 10 min in an automatic washing machine. Two additional bags per each feedstuff, containing non-incubated material, were also washed to determine the soluble fraction. After washing, bags were dried at 65 °C for 48 h, and the residues inside were stored in hermetic plastic bags until analysis.

Analysis of CP and NDF in the bags' residues was performed at the Laboratory of Animal Nutrition, Faculty of Veterinary Sciences, University of Zaragoza, Spain, following the same procedures used for feedstuffs.

The disappearance of DM, CP, and NDF from polyester bags on time was fitted to the first-order kinetic equation described by Ørskov and McDonald [13]:

$$y = a + b\,(1 - e^{-ct}),$$

where 'y' represents degradation at a given time of incubation ('t'), 'a' the soluble fraction in the rumen, 'b' the non-soluble but potentially degradable fraction, and 'c' the fractional rate of degradation of fraction 'b'. Potential degradability is represented by the sum of 'a' and 'b'.

2.3. Rumen Fermentation

The next day after the assessment of rumen degradability, samples of rumen liquid were taken right before, and then at 4, 8, 12, 16, and 24 h after accession of the animals to the pasture. Fluid (about 200 mL) was removed using a plastic hose (40 cm long and 0.8 cm internal diameter) connected to a 50 mL syringe. Representative samples were taken moving the tube in all directions inside the rumen while sampling. Then, rumen pH was immediately measured, in triplicate, using a portable pH-meter HACH, model HQ40d. After pH recording, fluid was strained through four layers of sterile gauze, and aliquots were taken, in duplicate, for analysis of ammonia (10 mL of rumen fluid in 13 mL plastic tubes containing 0.2 mL of 50% sulfuric acid). Sampling was repeated one month later, just before the last weighing of the animals. In this second sampling, volatile fatty acids (VFA) were also analyzed by duplicate (4 mL of rumen liquid in 5 mL plastic tubes containing 1 mL deproteinizing solution: 5 mL H_3PO_4 and 0.5 g methyl valeric acid (as internal standard) in 250 mL milli Q water). All samples were frozen at −20 °C until analysis.

Ammonia nitrogen concentration in rumen samples was assessed by the colorimetric method described by Weatherburn [23] at the Laboratory of Water, Faculty of Exact and Natural Sciences, National University of Asunción, Paraguay. The analysis of VFA was carried out at the University of Zaragoza following the procedures described by Gimeno et al. [24].

2.4. Statistical Analysis

Analysis of the variance of all variables was carried out using the SAS Software (SAS Inst. Inc., Cary, NC, USA, v 9.2). Data of final LW and ADG were analyzed with PROC GLM, following the model

$$y = \mu + TS_i + SL_j + TSSL_{ij} + \varepsilon_{k(ij)},$$

where TS_i represents the effect of the type of sward grazed, SL_j the effect of the supplementation level, $TSSL_{ij}$ their interaction, and $\varepsilon_{k(ij)}$ the experimental error. Type of sward, supplementation level, and their interaction were included in the model as fixed factors, and animal as random. Values were corrected by covariance using the initial body weight as a covariate.

Potential degradability and fractional rate of degradation of DM, CP, and NDF of the two swards were also analyzed with PROC GLM using the same model as above. The only difference was that the random effect was the animal within the type of sward and supplementation level. For the degradability of the expellers, the model used was

$$y = \mu + TS_i + SL_j + TE_k + TSSL_{ij} + TSTE_{ik} + SLTE_{jk} + TSSLTE_{ijk} + \varepsilon_{k(ij)},$$

where TS_i represents the effect of the type of sward grazed, SL_j the effect of the supplementation level, TE_k the type of expeller incubated, $TSSL_{ij}$ the interaction between type of sward and supplementation level, $TSTE_{ik}$ the interaction between type of sward and type of expeller, $SLTE_{jk}$ the interaction between supplementation level and type of expeller, $TSSLTE_{ijk}$ the triple interaction between type of sward, level of supplementation and type of expeller, and $\varepsilon_{k(ij)}$ the experimental error. All factors were included as fixed, whereas the random effect was the animal within the type of sward and supplementation level.

Values of pH and ammonia concentration in the rumen liquid were analyzed as repeated measures with the MIXED procedure, following the model

$$y = \mu + TS_i + SL_j + D_k + H_{l(k)} + TSSL_{ij} + TSD_{ik} + TSH_{il(k)} + SLD_{jk} + SLH_{jl(k)} + TSSLD_{ijk} + TSSLH_{ijl(k)} + \varepsilon_{m(ijkl)},$$

where TS_i represents the effect of the type of sward grazed, SL_j the effect of the supplementation level, D_k the sampling day, $H_{l(k)}$ the sampling time within a day, $TSSL_{ij}$ the interaction between type of sward and supplementation level, TSD_{ik} the interaction between type of sward and day of sampling, $TSH_{il(k)}$ the interaction between type of sward and time of sampling within a day, SLD_{jk} the interaction between supplementation level and day of sampling, $SLH_{jl(k)}$ the interaction between supplementation level and time of sampling within a day, $TSSLD_{ijk}$ the triple interaction between type of sward, level of supplementation, and sampling day, $TSSLH_{ijl(k)}$ the triple interaction between type of sward, level of supplementation and time of sampling within a day, and $\varepsilon_{m(ijkl)}$ the experimental error. All effects were included as fixed factors, whereas the random factor was the animal within the type of sward and supplementation level. Sampling time within a day was used as a repeated measure.

The total concentration of VFA in the rumen, and molar proportions of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate were also analyzed as repeated measures with the MIXED procedure. In this case, the model was

$$y = \mu + TS_i + SL_j + H_k + TSSL_{ij} + TSH_{ik} + SLH_{jk} + TSSLH_{ijk} + \varepsilon_{l(ijk)},$$

where TS_i represents the effect of the type of sward grazed, SL_j the effect of the supplementation level, H_k the sampling time, $TSSL_{ij}$ the interaction between type of sward and supplementation level, TSH_{ik} the interaction between type of sward and sampling time, SLH_{jk} the interaction between supplementation level and time of sampling, $TSSLH_{ijk}$ the triple interaction between type of sward, level of supplementation and time of sampling, and $\varepsilon_{l(ijk)}$ the experimental error. All effects were included as fixed factors, whereas the random factor was the animal within the type of sward and supplementation level. Sampling time was used as a repeated measure. For rumen fermentation variables, the variance–covariance structure was selected based on the lowest Akaike information criterion. Differences were considered significant if $p < 0.05$. A tendency was considered if $0.1 > p > 0.05$.

3. Results

3.1. Rumen Degradability of Pastures

Potential degradability of the DM and NDF of the two pastures was affected ($p < 0.05$) by the interaction between pasture type and supplementation level. For DM, this interaction was reflected in higher values for NS than S in sheep grazing NMS but not in those grazing CMS (Table 2). For NDF, the interaction reflected higher values for NS than S in sheep grazing NMS but the opposite in those grazing CMS. Sheep grazing CMS showed higher values than those grazing NMS for both DM and NDF regardless of the supplementation level. The potential degradability of CP was affected only by the type of pasture ($p < 0.0001$), with higher values for CMS (34.9% vs. 58.5%).

The fractional rate of degradation of DM was affected by the type of pasture ($p < 0.0001$; 0.030 h^{-1} vs. 0.040 h^{-1} for NMS and CMS, respectively) and level of supplementation ($p = 0.0030$; 0.038 h^{-1} vs. 0.032 h^{-1} for NS and S animals, respectively). The interaction between both factors affected CP's fractional rate of degradation. This interaction was the result of higher values for S than for NS (2.4-fold) in animals grazing NMS but not in those grazing CMS. Also, the fractional rate of degradation of CP in NS animals was

31% lower for those grazing NMS, whereas for S animals the lower values (32% lower) were for those grazing CMS. The fractional rate of degradation of NDF was affected by pasture type ($p < 0.0001$; 0.025 h^{-1} vs. 0.040 h^{-1} for NMS and CMS, respectively) and level of supplementation ($p = 0.0172$; 0.034 h^{-1} vs. 0.031 h^{-1} for NS and S, respectively).

Table 2. Potential degradability (a + b; g/100 g dry matter), and fractional rate of degradation (c; h^{-1}), of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of natural mixed swards (NMS) or cultivated monophytic swards (CMS) of *Brachiaria brizantha* cv. Marandu, non-supplemented (NS) or supplemented (S) with 1 kg dry matter/100 kg live weight of a mixture of 1/3 of endosperm expeller and 2/3 of mesocarp expeller from Acrocomia aculeata (Jacq.) Lodd. ex Mart.

Pasture (P)		**NMS**		**CMS**			***p*-Value**		
Supplementation (S)		**NS**	**S**	**NS**	**S**	**SEM**	***p***	**S**	***p* × S**
DM	a + b	44.6 bA	39.9 aA	68.7 B	70.7 B	1.11	<0.0001	0.13	0.003
	c	0.034	0.026	0.041	0.038	0.0019	<0.0001	0.003	0.064
CP	a + b	32.7	37.1	54.8	62.3	4.60	<0.0001	0.10	0.65
	c	0.070 aA	0.168 bB	0.102 B	0.115 A	0.0133	0.32	0.0004	0.002
NDF	a + b	42.9 bA	33.9 aA	63.8 aB	68.1 bB	1.43	<0.0001	0.049	0.0002
	c	0.026	0.024	0.041	0.038	0.0013	<0.0001	0.017	0.60

SEM: standard error of the mean; a, b: different lower-case letters indicate differences between supplementation levels within a pasture type at $p < 0.05$; A, B: different upper-case letters indicate differences between types of pasture within a supplementation level at $p < 0.05$.

3.2. Rumen Degradability of Expellers

The potential degradability of DM and CP of both ENE and ME was not affected by the type of pasture or the level of supplementation ($p > 0.05$). There were also no differences between ENE and ME (Table 3). However, the fractional rate of degradation of DM was faster ($p = 0.007$) for ME (0.129 h^{-1}) than for ENE (0.044 h^{-1}). The potential degradability of NDF was higher ($p < 0.0001$) for ENE (72.5%) than for ME (54.4%) with no differences in fractional rate of degradation ($p > 0.1$). None of the interactions between pasture type, level of supplementation, and expeller type, were significant ($p = 0.20$).

Table 3. Potential degradability (a + b; g/100 g dry matter), and fractional rate of degradation (c; h^{-1}), of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of mesocarp (ME) and endosperm (ENE) expellers from Acrocomia aculeata (Jacq.) Lodd. ex Mart. incubated in the rumen of sheep grazing natural mixed swards (NMS) or cultivated monophytic swards (CMS) of *Brachiaria brizantha* cv. Marandu, non-supplemented (NS) or supplemented (S) with 1 kg dry matter/100 kg live weight of a mixture of 1/3 ENE and 2/3 ME.

Pasture (P)		**NMS**				**CMS**								
Supplementation (S)		**NS**		**S**		**NS**		**S**				***p*-Value**		
Expeller (E)		**ME**	**ENE**	**ME**	**ENE**	**ME**	**ENE**	**ME**	**ENE**	**RSD_1**	**RSD_2**	***p***	**S**	**E**
DM	a + b	81.0	81.5	79.2	69.7	78.9	82.9	80.6	82.1	2.94	7.32	0.20	0.21	0.72
	c	0.072	0.054	0.130	0.043	0.150	0.035	0.165	0.045	0.0049	0.0696	0.33	0.46	0.007
CP	a + b	83.9	87.7	81.3	63.3	76.4	84.7	79.6	89.1	9.45	15.76	0.39	0.21	0.82
	c	0.037	0.044	0.044	0.027	0.131	0.033	0.269	0.032	0.1291	0.1373	0.10	0.50	0.073
NDF	a + b	59.2	75.3	51.5	65.2	51.0	75.8	55.0	73.7	3.87	6.83	0.63	0.11	<0.0001
	c	0.049	0.079	0.117	0.059	0.123	0.055	0.081	0.068	0.0622	0.0552	0.77	0.81	0.20

RSD_1, residual standard deviation for comparison between pasture types, levels of supplementation, and their interaction; RSD_2, residual standard deviation for comparison between expellers and their interactions.

3.3. Animal Performance

Final LW was affected by the interaction between sward type and supplementation level ($p = 0.0334$), with no differences between supplementation levels for animals grazing CMS, but higher values for supplemented vs. non-supplemented sheep grazing NMS (Table 4). No differences were found for the type of pasture grazed. A marked positive effect of supplementation on ADG ($p = 0.007$) was evident. Supplemented animals grazing

NMS grew, on average, nearly 90 g/d more than non-supplemented animals (more than 6-fold), whereas the increment was less than 25 g/d in animals consuming CMS (40% higher in supplemented animals).

Table 4. Final live weight (FLW) and average daily gain (ADG) of sheep grazing natural mixed swards (NMS) or cultivated monophytic swards (CMS) of *Brachiaria brizantha* cv. Marandu, non-supplemented (NS) or supplemented (S) with 1 kg dry matter/100 kg live weight of a mixture of 1/3 of endosperm expeller and 2/3 of mesocarp expeller from Acrocomia aculeata (Jacq.) Lodd. ex Mart.

Pasture (P)	**NMS**		**CMS**		**SEM**	***p*-Value**		
Supplementation (S)	**NS**	**S**	**NS**	**S**		***p***	**S**	***p* × S**
FLW (kg)	37.5 a	41.7 b	39.0	39.8	0.69	0.54	0.005	0.033
ADG (g/d)	17	104	58	81	15.5	0.75	0.007	0.066

SEM: standard error of the mean; a, b: different letters indicate differences between supplementation levels within a type of pasture at $p < 0.05$.

3.4. Rumen Fermentation

Sheep consuming NMS showed higher (2.2% on average) rumen pH ($p = 0.028$) than those grazing CMS (Table 5), and supplementation tended ($p = 0.056$) to reduce rumen pH. Animals grazing CMS tended to have a higher concentration of VFA in the rumen than those grazing NMS ($p = 0.09$). The molar proportion of valerate was also 84% higher ($p = 0.0055$) in CMS animals. For the rest of the variables, there was no effect ($p > 0.1$) of either the type of pasture grazed or the level of supplementation. The interaction between these two factors was not significant ($p > 0.1$) in any case.

Table 5. Average daily rumen pH, daily average concentrations of volatile fatty acids (VFA; mmol/L) and ammonia (mg/100 mL), and daily average molar percentage (mmol/100 mmol) of the main VFA in the rumen fluid of sheep grazing natural mixed swards (NMS) or cultivated monophytic swards (CMS) of *Brachiaria brizantha* cv. Marandu, non-supplemented (NS) or supplemented (S) with 1 kg dry matter/100 kg live weight of a mixture of 1/3 of endosperm expeller and 2/3 of mesocarp expeller from *Acrocomia aculeata* (Jacq.) Lodd. ex Mart.

Pasture (P)	**NMS**		**CMS**			***p*-value**		
Supplementation (S)	**NS**	**S**	**NS**	**S**	**SEM**	***p***	**S**	***p* × S**
pH	6.51	6.41	6.38	6.25	0.043	0.028	0.056	0.74
Total VFA	53.3	51.7	89.7	76.5	19.44	0.090	0.62	0.69
Ammonia	5.90	6.66	11.29	8.04	1.643	0.11	0.49	0.29
Acetate	56.9	57.9	52.4	53.5	3.62	0.16	0.70	0.98
Propionate	12.2	10.5	15.5	14.1	2.41	0.11	0.42	0.94
Butyrate	4.89	5.07	5.54	5.38	1.745	0.72	0.99	0.90
Isobutyrate	0.853	0.958	0.895	0.731	0.3431	0.72	0.91	0.61
Valerate	0.309	0.433	0.653	0.709	0.0804	0.0055	0.19	0.59
Isovalerate	0.850	1.111	1.008	0.833	0.4691	0.87	0.90	0.55

SEM: standard error of the mean.

The interaction between supplementation level and time of sampling affected the molar proportions of propionate ($p = 0.02$) and butyrate ($p = 0.032$). The proportion of propionate was higher in NS than in S animals at all times except at 4 h after accession to the pasture. The proportion of butyrate was higher in NS than in S animals at 8 and 12 h, but lower at 4, 16, and 24 h.

4. Discussion

According to their chemical composition (Table 1), both NMS and CMS can be classified as pastures of relatively poor quality. Their content in CP was below 70 g/kg DM, considered the threshold for an adequate activity of rumen microorganisms, and for maintaining an adequate intake [25]. In addition, the amount of structural carbohydrates was high, and typical of tropical grasses (Table 1).

With respect to the expellers from *Acrocomia aculeata* (Jacq.) Lodd. ex Mart., both had a content of residual oil (EE in Table 1) higher than 15% because of the mechanical process of extraction. This makes them a suitable energetic supplement. In addition, ENE showed a high concentration in protein (34.4%), comparable to other expellers from oleaginous plants, which makes it also a suitable protein supplement. The mixture of one-third ENE and two-thirds ME showed a CP content similar to commercial concentrates for grazing sheep in Paraguay (*ca*. 14% on a dry matter basis). At a daily rate of 1% LW, the protein content of the mixture is sufficient to maintain grazing sheep with characteristics similar to those of the animals used in the present experiment, and even to allow moderate growth rates (50–100 g/day).

We are aware that the low number of experimental units (sheep) used in the present trial may compromise the veracity, repeatability, and reliability of our results. This low number was obliged by the on-farm tropical conditions of the experiment, as maintaining cannulated sheep alive in the wet tropical conditions of the study is especially difficult. However, many variables reached statistical significance and this indirectly indicates that the low number of animals was not a limitation in this study. Undoubtedly, future studies should be based on more powerful statistical designs.

4.1. Rumen Degradability

The lower potential degradability and fractional rate of degradation of NMS compared to CMS (Table 3) could be explained by the higher content in NDF, ADF, and ADL of the former (Table 1). To this respect, Van Soest [26] pointed out that rumen digestion of a determined ingredient or combination of them is negatively affected by its NDF content, and also by the degree of lignification of the ADF fraction. The potential degradability of DM of CMS was lower than that reported (79.4%) by Lopes et al. [27], but it must be taken into account that these authors worked with unsupplemented cattle instead of sheep, and that their pasture had a higher content in CP, which may have helped the rumen microorganisms to ferment more. Unfortunately, we have not been able to find published papers dealing with the rumen degradability of different pastures in sheep subjected to varying feeding conditions (e.g., forage-to-concentrate ratio). In our experiment, the potential degradability of the DM of CMS was not affected by the supplementation level, as previously stated by Oliveira et al. [28] in cattle. However, supplementation negatively affected the fractional rate of degradation of the DM of both NMS and CMS, probably due to the high EE level of the supplement. The fractional rate of degradation of the DM of CMS in our experiment was higher than that found by Lopes et al. [27] or Oliveira et al. [28] in non-supplemented cattle but huge variations may be found in pasture quality according to soil characteristics or climate conditions. Between-species differences (cattle vs. sheep) are also expected.

Regarding CP degradation of the pastures in our experiment, the potential degradability may be considered moderate. However, our values were lower than those reported by Lopes et al. [27] or Ibrahim et al. [29], both in cattle. Again, differences in animal species and feeding conditions might account for at least part of the dissimilarities. On the other hand, the fact that supplementation increased the fractional rate of degradation of CP in sheep grazing NMS but not in those grazing CMS is probably due to the lower ME content of the former (Table 1). We can speculate that bacteria in the rumen of NMS sheep had a less favorable environment, in terms of energy availability, than those in the rumen of CMS sheep. Then, the energy boost provided by the supplement would have had a more intense effect on sheep grazing NMS than on those grazing CMS. Lopes et al. [27] reported 3–4-fold lower values of fractional rate of degradation of CP in cattle grazing CMS than those reported in the present experiment, whereas Oliveira et al. [28] and Ibrahim et al. [29] had intermediate values. Between-species differences, together with diverse environmental and feeding conditions are surely responsible for the dissimilarities.

The potential degradability of NDF of the pastures in the present experiment was increased by supplementation in animals grazing CMS but was decreased in sheep grazing

NMS (Table 2). Even though microbiome studies were not carried out in the present work, a likely explanation could be a change in bacterial populations, which could have been modified as a result of different factors, including the potential presence of bioactive compounds in the supplement (e.g., phenolic compounds, vitamin C, or β-carotene/vitamin A; Oliveira et al. [30]). The values of potential degradability of NDF of CMS presented in this work were similar to those reported by Lopes et al. [27] in cattle, and intermediate between those found by Oliveira et al. [28] and Ibrahim et al. [29], also in cattle. With respect to the fractional rate of degradation of NDF of CMS, our values were similar to those given by Ibrahim et al. [29] but higher than those found by Lopes et al. [27] or Oliveira et al. [28] in cattle.

It can be argued that the feeding behavior, metabolism, and physiology of cattle are different from those of sheep; hence, a comparison of our results to others obtained from the former species would be useless. Unfortunately, we were not able to find specific information in the literature dealing with the rumen degradability of *Brachiaria brizantha* cv. Marandu or the natural pastures used in the present work, in sheep subjected to varying feeding conditions (e.g., pasture-to-concentrate ratio). An alternative could have been to compare our results to those obtained in sheep fed different pastures, but in our opinion, the plant species has a greater impact on rumen degradation than the animal species consuming them.

On the other hand, it appears that even in grazing cattle, the feeding conditions (e.g., supplementation vs. non-supplementation) greatly affect the degradation of the pastures. The obvious conclusion then is that both potential degradability and fractional rate of degradation of grazed pastures should be assessed in each determined feeding system.

With respect to the grugru palm expellers, no information has been found in the literature with respect to their degradation in the rumen. Hence, the results of the present experiment will be compared to rumen degradation variables of other ingredients usually included in the concentrates given to grazing sheep in the area of our study. As an example, the average potential degradability of DM of ENE (78.9%) was lower than that reported by Sauvant et al. [31] for soybean meal (97%) or palm kernel meal (92%) but similar to that of sunflower meal (78%) or copra meal (83%). On the other hand, the average fractional rate of degradation of DM of ENE (0.044 h^{-1}) was lower than that reported by Sauvant et al. [31] for soybean meal (0.080 h^{-1}), sunflower meal (0.085 h^{-1}), or copra meal (0.150 h^{-1}), but similar to that of palm kernel meal (0.040 h^{-1}). Similar comparisons can be made for CP and NDF of ENE, or for DM, CP, and NDF of ME ([32,33]). Even in the case of CP of ENE, other authors [34] found comparable or higher values with soybean meal or faba bean, respectively. In summary, ENE has the advantages of sunflower meal in terms of protein content and its potential degradability, but has a slower degradation rate. As a result, this by-product could be used when slow-degraded carbohydrates are abundant in the diet or when the flow of undegraded protein to the intestines is desirable. Of course, the competitive price between ENE and other protein supplements should be considered. In the case of ME, its use as a unique supplement would be limited by its low protein content. Assuming additivity for potential degradability and fractional rate of degradation, the use of a mixture of one-third ENE plus two-thirds ME as a protein supplement seems useful in grazing sheep.

4.2. Effects of Grazed Pasture and Supplementation on Animal Performance

In the present work, individual intake of concentrate was not assessed and pasture intake was not measured so the only available traits of animal performance were final weight and ADG.

Final LW was increased with supplementation in animals grazing NMS but not in those consuming CMS, whereas ADG was enhanced more substantially in the first group (Table 2). As stated above, we can speculate that bacteria in the rumen of NMS sheep had a less favorable environment, in terms of energy availability, than those in the rumen of CMS sheep. Then, the energy boost provided by the supplement would have had a more intense effect on sheep grazing NMS than on those grazing CMS.

In sheep grazing natural pastures with different levels of supplementation (40.4% ground corn, 56.6% soybean meal, and 3% minerals), Dantas et al. [35] also found increases in LW when the animals were supplemented at 0%, 1%, and 1.5% LW. In the same way, Arias and Ocampos [15] recorded higher ADG in lambs grazing natural pastures supplemented with 250 g of commercial concentrate compared to non-supplemented animals.

With respect to CMS, Carvalho et al. [16] were not able to find differences in final LW between sheep grazing *Brachiaria brizantha* cv. Marandu with different types of supplementation (mineral mix, NaCl plus ground corn, mineral mix plus urea/ammonium sulfate (9:1), NaCl, ground corn plus soybean meal, or mineral mix plus urea/ammonium sulfate (9:1) plus NaCl plus soybean meal). Similarly, Oliveira et al. [36] did not observe differences in final LW between supplemented (with 180 g DM/day of supplements containing ground corn, wheat meal, and either cotton cake, soybean meal, or urea) or non-supplemented sheep grazing *Cynodon dactylon* (L.) Pers. Voltolini et al. [37] also failed to find differences in final LW between sheep grazing *Cenchrus ciliaris* L. supplemented at different levels (0%, 0.33%, 0.66%, and 1% LW, on a dry matter basis, of a mixture of ground corn, wheat bran, soybean meal, and urea) or non-supplemented. On the contrary, Almeida et al. [38] recorded higher final LW in supplemented (mixture of ground corn, soybean meal, Mesquite pod meal, wheat meal, sorghum meal, and urea) ewes grazing *Urochloa mosambicensis* (Hack.) Dandy compared to non-supplemented animals. It must be considered that the different trials were performed in different agronomic and climatic conditions, and with different animal species and stocking rates, but the positive effect of supplementation was much higher in terms of ADG and final LW in animals grazing multi-species pastures of low quality than in those consuming high-quality mono-species swards. This outcome was also obtained in the present experiment.

Regarding specific supplementation with *Acrocomia aculeata* (Jacq.) Lodd. ex Mart by-products, the available information is very scarce. Azevedo et al. [6] report the results of an experiment with confined male lambs receiving a complete diet (300 g sorghum silage and 700 g concentrate per kg DM) with different proportions of grugru palm cake (a mixture of unknown proportions of ENE and ME). The concentrate was formulated with corn, soybean meal, cottonseed hulls, and 0, 100, 200, or 300 g grugru palm cake/kg DM. These authors did not find an effect of including grugru palm in the concentrate on final LW or ADG, probably due to the high quality of the forage. In the present paper, supplementation with a mixture of one-third ENE and two-thirds ME, at a daily rate of 1% LW on a DM basis, enhanced ADG in sheep grazing NMS or CMS, more pronouncedly in the former. To the best of our knowledge, no information has been published regarding the animal performance of grazing sheep supplemented with grugru palm cake, and hence our results can be considered original.

In terms of economic profitability, sheep production based on pasture is advantageous due to lower production costs compared to systems that are more intensive. However, grazing systems are limited in terms of productive efficiency, and supplementation may increase the profitability of the farm by allowing faster rates of growth, as in the present work, and reducing the time to slaughter [39]. Nevertheless, the cost of the supplement must be considered as it could exceed the expected benefits. In our case, the cost of the supplement provided by Industrial Aceitera SACI was 135 EUR/t, whereas one kg of lamb was paid at EUR 3.13 in the local market at the time of the trial. With these figures, the extra ADG of supplemented animals represented 0.282 and 0.078 EUR/animal and day for animals grazing NMS and CMS, respectively. As the cost of supplementation was the same (0.049 EUR/animal and day), this practice was economically profitable regardless of the type of pasture consumed.

4.3. Effects of Grazed Pasture and Supplementation on Rumen Environment

Rumen pHs were within the range of values considered normal for ruminants grazing tropical pastures, and the higher figures for animals consuming NMS vs. CMS ($p = 0.028$) were matched with a lower concentration ($p = 0.09$) of total VFA (Table 5). No publications have been found dealing with rumen fermentation variables in grazing ruminants supplemented with a mixture of one-third ENE and two-thirds ME. As a result, the discussion has been focused on the comparison of our results with those obtained in trials carried out with tropical pastures either supplemented or non-supplemented.

Muinga et al. [40] did not find differences in pH, VFA, or ammonia concentrations in the rumen of steers fed either *Cenchrus purpureus* (Scumach.) Morrone alone or supplemented with one or two kg of *Leucaena leucocephala* (Lam.) de Wit or with *Leucaena leucocephala* (Lam.) de Wit plus 1 kg maize bran per animal (on a dry matter basis). The range of VFA concentrations was 75–86 mmol/L, similar to the values obtained in the present work for CMS either supplemented or non-supplemented. In the paper by Muinga et al. [40], ammonia concentration ranged between 15.2 mg/100 mL and 25.2 mg/100 mL, and pH varied between 6.42 and 6.95, values slightly higher than those found in the present work. In our research, the average ammonia concentration was higher than 5 mg/100 mL, the threshold suggested by Satter and Slyter [41] for the correct functioning of rumen bacteria. Foster et al. [42] did not find differences in pH, ammonia, or VFA concentration, and molar proportions of the different VFA in the rumen of lambs fed *Paspalum notatum* Flüggé hay with or without supplementation with soybean meal (4.25% of the ration DM). On the other hand, Morais et al. [43], in a trial with cattle grazing *Brachiaria brizantha* cv. Marandu supplemented with a commercial compound feed at 0.5% LW, reported average ammonia concentrations of 13.12 mg/100 mL, higher than that obtained in the present work. Morais et al. [43] also found higher VFA concentrations than in the present work. Manella et al. [44] observed VFA concentration values between 55.9 mmol/L and 71.4 mmol/L in cattle grazing *Brachiaria brizantha* cv. Marandu and supplemented with either a protein concentrate or *Leucaena leucocephala* (Lam.) de Wit, with these values being lower than those reported in the present experiment. Also, ammonia concentrations reported by Manella et al. [44] were lower (between 1.7 mg/100 mL and 2.2 mg/100 mL) than those found in our trial. Carvalho et al. [16] measured the rumen pH of sheep grazing *Brachiaria brizantha* cv. Marandu and supplemented with various types of ingredients (minerals, energy, energy–protein, and protein) and also found no differences between treatments. These authors measured also ammonia concentration, which was higher in the protein- and protein–energy-supplemented animals. Their values for these treatments were also higher than those obtained in the present work.

In our work, supplementation of sheep grazing either NMS or CMS with a mixture of one-third ENE and two-thirds ME did not affect variables defining rumen fermentation. However, grazing CMS produced a numerically lower acetate/propionate ratio than grazing NMS (5.09 vs. 3.59), whereas supplementation increased that ratio (4.02 vs. 4.65 for non-supplemented and supplemented animals, respectively). The production of propionic incorporates 2H, competing as a sink with CH_4 [45] so, in the absence of an economic study, supplementation of sheep grazing either NMS or CMS with a mixture of one-third ENE and two-thirds ME does not seem to be a good strategy to reduce the emissions of methane from this type of production. The high-fat content of the supplement (166 g/kg DM) likely produced a shift in the microbiome [46].

5. Conclusions

Assuming additivity for the potential degradability and fractional rate of the degradation of ENE and ME, the use of a mixture of one-third ENE plus two-thirds ME as a protein supplement seems useful in sheep grazing either multi-species natural pastures or monophytic swards of *Brachiaria brizantha* cv. Marandu. Such supplementation slows down the fractional rate of degradation of the pastures, the effect of which is more intense in animals grazing natural swards. Due to the effects of the pasture grazed and level of supplementation, rumen degradation of grugru palm expellers should be carried out in each specific feeding schedule in grazing sheep.

Supplementation with a mixture of one-third ENE and two-thirds ME from *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. to sheep grazing either multi-species natural pastures or monophytic swards of *Brachiaria brizantha* cv. Marandu increases average daily gain, more substantially with multi-species natural pastures. Hence, this type of supplementation could be recommended especially in animals grazing low-quality pastures.

Grazing CMS seems to decrease the acetate/propionate ratio compared to grazing NMS, whereas supplementation with grugru palm by-products at 1 kg dry matter/100 kg live weight seems to increase that ratio. Supplementation of sheep grazing CMS with a mixture of one-third ENE and two-thirds ME, therefore, does not seem to be a good strategy for reducing the emissions of methane from this type of production.

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