

Article

Enhancing Rumen-Undegradable Protein via Processing Techniques in a Dual-Flow Continuous Culture System

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Abstract: The use of processing techniques to increase the rumen-undegradable protein (RUP) content of protein meals aims to enhance the nutritional performance of high-performance animals. This study evaluated the effects of various processing techniques applied to peanut and cottonseed meals on ruminal parameters using a dual-flow continuous culture system. These two feeds were individually analyzed in two experiments, each one using five fermenters (1297 ± 33 mL) in a 5 × 5 Latin square arrangement with five periods of 10 d each, with 7 d for diet adaptation and 3 d for sample collections. Five treatments were evaluated in each experiment: no processed meal (control); meal thermally treated in an autoclave with xylose (autoclave); meal thermally treated in a conventional oven with xylose (oven); meal thermally treated in a microwave with xylose (microwave); and meal treated with tannin (tannin). All diets contained 60% concentrate (corn, minerals, and processed meal). Fermenters were fed 55 g of dry matter per day, divided equally into two meals at 06:00 and 18:00 h. The solid and liquid dilution rates were adjusted daily to 5.5% and 11% per hour, respectively. On days 8, 9, and 10, 500 mL samples of solid and liquid digesta effluent were collected, mixed, homogenized, and stored at −20 °C. Subsamples of 10 mL were preserved with 0.2 mL of a 50% H₂SO₄ solution for later determination of NH₃-N and volatile fatty acids. Microbial biomass was isolated from the fermenters for chemical analysis at the end of each experimental period. Data were analyzed using the MIXED procedure in SAS with a significance level of $\alpha = 0.05$. Regarding cottonseed meal, the tannin treatment tended to have a lower true digestibility of DM compared to the control, autoclave, and oven treatments ($p = 0.09$). Additionally, tannin fermenters exhibited a lower apparent digestibility of CP compared to all other treatments ($p = 0.04$). The tannin and microwave treatments resulted in the highest flow of dietary nitrogen and the lowest supply of RDP-N ($p < 0.01$). The control treatment had a greater flow of NH₃-N compared to other treatments ($p < 0.01$). Regarding peanut meal, the autoclave and tannin treatments exhibited the highest acetate concentration ($p = 0.03$) and the lowest apparent digestibility of CP ($p < 0.01$). The tannin treatment increased the RUP content without impairing ruminal fermentation and exhibited a greater supply of RDP-N compared to all other treatments ($p = 0.02$). No significant differences were observed for the other digestibility and fermentation parameters ($p > 0.20$). Our results indicate that tannin inclusion and microwave processing were the most effective methods for reducing the protein fraction available in the rumen for cottonseed meal. Additionally, tannin inclusion increased the RUP in peanut meal without negatively affecting ruminal fermentation.



Academic Editor: Cláudia Braga Pereira Bento

Received: 29 November 2024

Revised: 27 January 2025

Accepted: 1 February 2025

Published: 12 February 2025

Citation: Loregian, K.E.; Silva, M.J.; Dourado, S.B.; Guimarães, J.; Amâncio, B.R.; Magnani, E.; Silva, T.H.; Branco, R.H.; Benedeti, P.D.B.; Paula, E.M. Enhancing Rumen-Undegradable Protein via Processing Techniques in a Dual-Flow Continuous Culture System. *Fermentation* **2025**, *11*, 94. <https://doi.org/10.3390/fermentation11020094>

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Keywords: beef cattle; cottonseed meal; nitrogen; peanut meal

1. Introduction

Protein is a crucial nutrient for ruminants, and it is divided into rumen-undegradable protein (RUP) and rumen-degradable protein (RDP). Their combination ensures the appropriate and efficient production of metabolizable protein necessary for both beef and dairy cattle [1,2]. Additionally, RUP is the fraction of dietary protein that is digested in the abomasum and small intestine of ruminants, contributing significantly to the pool of metabolizable amino acids, especially for high-performance and young animals [3]. For these animals, microbial protein (MP) alone is insufficient to meet their amino acid requirements [1,2,4]. Therefore, increasing the RUP content from protein sources can be an alternative to improve animal performance.

Increasing the protein fraction available to post-rumen compartments (abomasum and small intestine) can enhance the flow of amino acids to the intestine [5]. Furthermore, the physical or chemical processing of feed significantly influences its digestibility [6]. Studies have shown that processing methods such as tannin treatment, xylose treatment, and heat application via a microwave, conventional ovens, or autoclaving can increase the RUP content of feed [7–10]. However, amino acids exhibit varying digestibility and absorption rates, which depend on the intensity and duration of the feed's exposure to these processing methods [5,11]. Therefore, understanding the processing techniques used in feed preparation is crucial for effective diet formulation. This approach not only aims for cost efficiency but also improves nitrogen use efficiency, aligning with environmental policies [2,12].

Soybean meal is a primary protein source in beef cattle diets due to its excellent amino acid profile and well-established procedures to protect its protein from rumen fermentation [13]. However, its high cost has prompted the exploration of alternative protein sources, such as peanut and cottonseed meals [14–17]. Recent studies have evaluated and proposed optimal conditions for processing cottonseed and peanut meals using heat treatments (autoclave, conventional, and microwave ovens), tannin, and xylose to increase RUP [8–10]. These studies have used in situ and in vitro techniques [18,19] to evaluate two protein sources: cottonseed meal (*Gossypium* spp.) and peanut meal (*Arachis hypogaea*). The meals were subjected to the following processing methods: untreated samples, used as control treatments; autoclaving at 127 °C with an internal pressure of 117 kPa for different durations (8, 16, and 24 min), with and without the addition of xylose (2%); oven roasting at 150 °C for different durations (30, 60, and 90 min), with and without the addition of xylose (2%); microwave heating for different durations (2, 4, and 6 min), with and without the addition of xylose (2%); and three different concentrations of tannin (Tanfeed® with a minimum of 70% tannin content; condensed tannin from *Acacia mearnsii*, 85% condensed and 15% hydrolyzed, Tanac SA, Montenegro, RS, Brazil): 0, 2, 4, and 6% of dry matter (DM) in the meal. In these studies, tannin inclusion and microwave heating have demonstrated superiority in enhancing RUP.

For the present study, we selected the eight best treatments (four for cottonseed meal and four for peanut meal) based on the four processing methods (autoclave, oven, microwave, and tannin). These selections were made according to the results of in situ degradation kinetics and in vitro intestinal digestibility of protein from the two protein sources, where the treatments selected were those with the highest RUP without affecting digestibility. The selected treatments were then individually analyzed in two separate experiments (one for cottonseed meal and one for peanut meal) using a dual-flow continuous

culture system on total mixed diets. Therefore, this study aimed to evaluate the effects of including peanut and cottonseed meals (processed with these techniques to increase RUP) in diets on ruminal parameters using a dual-flow continuous culture system. We hypothesize that the inclusion of these high RUP processed feeds could modify nitrogen flow by reducing the rumen-degradable protein (RDP) supply without compromising digestibility and other ruminal fermentation parameters.

2. Materials and Methods

2.1. Location, Ethical Approval, and Preliminary Study

The experiments were conducted at the Instituto de Zootecnia, Beef Cattle Research Center, Sertãozinho, São Paulo, Brazil. This study was carried out in strict accordance with the recommendations of the Animal Use Ethics Committee of the Instituto de Zootecnia. Animal care and the handling protocol were approved by this committee (Protocol Number 249-19).

A preliminary study was conducted to assess different techniques aimed at increasing the RUP content in commonly used protein sources in ruminants' diets. This study evaluated two protein sources: cottonseed meal (*Gossypium* spp.) and peanut meal (*Arachis hypogaea*) [8,9]. The meals were subjected to the following processing methods: (a) untreated samples, used as control treatments; (b) autoclaving at 127 °C with an internal pressure of 117 kPa for different durations (8, 16, and 24 min), with and without the addition of xylose (2%); (c) oven roasting at 150 °C for different durations (30, 60, and 90 min), with and without the addition of xylose (2%); (d) microwave heating for different durations (2, 4, and 6 min), with and without the addition of xylose (2%); and (e) three different concentrations of tannin (Tanfeed® with a minimum of 70% tannin content; condensed tannin from *Acacia mearnsii*, 85% condensed and 15% hydrolyzed, Tanac SA, Montenegro, RS, Brazil): 0, 2%, 4%, and 6% of DM in the meal [10]. In situ and in vitro techniques were used to determine RUP and simulate the intestinal digestibility of protein [18,19].

For the present study, we selected the eight best treatments (four for cottonseed meal and four for peanut meal) based on the four processing methods (autoclave, oven, microwave, and tannin). These treatments are detailed in the following treatment descriptions in Section 2.2. These selections were made according to the results of in situ degradation kinetics and in vitro intestinal digestibility of protein from the two protein sources. The selected treatments were then individually analyzed in two separate experiments (one for cottonseed meal and one for peanut meal) using a dual-flow continuous culture system on total mixed diets (present study).

2.2. Experimental Designs and Diets

For both experiments, we employed five dual-flow continuous culture fermenters, each with a working volume of 1297 ± 33 mL (model ENG RM-1; Engco LTDA, Piracicaba, SP), similar to the system described in the literature by Hoover, Crooker, and Sniffen [20].

These fermenters were utilized over five consecutive 10-day periods, allowing 7 days for adaptation and 3 days for sampling. The experimental setup followed a 5×5 Latin square design, with five replicates per treatment in each experiment. Thus, for this study, we conducted two experiments with five treatments each. We selected the eight best treatments (four for cottonseed meal and four for peanut meal) based on four processing methods (autoclave, oven, microwave, and tannin), plus a control treatment for each meal.

Experiment 1 (cottonseed meal) was conducted to evaluate the following five treatments: 1—conventional cottonseed meal (control); 2—cottonseed meal thermally treated in an autoclave with 2% xylose for 16 min (autoclave); 3—cottonseed meal thermally treated in a conventional oven with 2% xylose for 90 min (conventional oven); 4—cottonseed meal thermally treated in a microwave with 2% xylose (Sigma Aldrich, St Louis, MO, USA) for

6 min (microwave); and 5—cottonseed meal treated with 4% tannin (tannin). Details of the processing methods can be found in Molosse et al. [8], and details regarding the tannin methods can be found in Loregian et al. [10].

For experiment 2 (peanut meal), the following five treatments were evaluated: 1—conventional peanut meal (control); 2—peanut meal thermally treated in an autoclave with 2% xylose for 24 min (autoclave); 3—peanut meal thermally treated in a conventional oven with 2% xylose for 60 min (conventional oven); 4—peanut meal thermally treated in a microwave with 2% xylose (Sigma-Aldrich, St Louis, MO, USA) for 6 min (microwave); and 5—peanut meal treated with 6% tannin (tannin). Details of the processing methods can be found in Rigon et al. [9], and details regarding the tannin methods can be found in Loregian et al. [10].

The compositions of the processed meals are presented in Table 1. The diets consisted of Brachiaria hay, ground corn, minerals, and the respective processed or unprocessed cottonseed or peanut meals. Table 2 shows the chemical composition of the experimental diets used in both experiments.

Table 1. Chemical composition of processed ingredients in experiments 1 (cottonseed meal) and 2 (peanut meal).

Item ¹	Cottonseed Meal ²					Peanut Meal ³				
	Control	Autoclave	Oven	Microwave	Tannin	Control	Autoclave	Oven	Microwave	Tannin
DM, g/kg	930	925	930	961	793	928	921	966	922	684
OM, g/kg DM	855	855	859	890	716	836	826	874	829	578
CP, g/kg DM	543	517	533	518	463	456	447	457	454	374
RUP, g/kg CP	298	452	666	430	460	482	605	631	608	557
NDF, g/kg DM	355	216	228	272	338	336	418	424	380	289
EE, g/kg DM	4.40	6.90	5.60	8.40	8.90	23.0	16.3	18.2	22.1	19.3

¹ DM = dry matter; OM = organic matter; CP = crude protein; RUP = rumen-undegradable protein; NDF = neutral detergent fiber; EE = ether extract. ² Control = conventional cottonseed meal (CM); autoclave = CM treated with 2% xylose and heated in an autoclave for 16 min; oven = CM treated with 2% xylose and heated in a conventional oven for 90 min; microwave = CM treated with 2% xylose and heated in a microwave oven for 6 min; tannin = CM treated with 4% tannin. ³ Control = conventional peanut meal (PM); autoclave = PM treated with 2% xylose and heated in an autoclave for 24 min; oven = PM heat treated with 2% xylose and heated in a conventional oven for 60 min; microwave = PM treated with 2% xylose and heated in a microwave oven for 6 min; tannin = PM treated with 6% tannin.

Table 2. Chemical composition of diets in experiments of cottonseed and peanut meal.

Item ¹	Cottonseed Meal					Peanut Meal				
	Control	Autoclave	Oven	Microwave	Tannin	Control	Autoclave	Oven	Microwave	Tannin
Ingredient, g/kg										
Hay	400	400	400	400	400	400	400	400	400	400
Dry ground corn	379	367	374	368	328	331	325	332	330	260
Cottonseed meal ²	201	213	206	212	252	-	-	-	-	-
Peanut meal ²	-	-	-	-	-	249	255	248	320	320
Mineral mixture ³	20	20	20	20	20	20	20	20	20	20
Composition										
DM, g/kg	911	910	915	917	882	914	912	926	912	922
OM, g/kg DM	930	935	930	932	928	919	923	925	927	920
CP, g/kg DM	163	163	163	163	166	163	163	163	182	163
RUP, g/kg PB	363	465	610	451	469	484	570	588	578	536
NDF, g/kg DM	409	382	384	394	416	415	437	436	436	414
EE, g/kg DM	1.8	1.8	1.8	1.9	1.8	2.1	2.0	2.0	2.3	2.0

¹ DM = dry matter; OM = organic matter; CP = crude protein; RUP = rumen-undegradable protein; NDF = neutral detergent fiber; EE = ether extract. ² The respective processed meal was used for each treatment. ³ Provided (per kg of DM): Ca (min) = 160 g; Ca (max) = 70 g; P (min) = 80 g; moisture (max) = 20 g; Na (min) = 120 g; Cl (min) = 155 g; F (max) = 1.5 mg; Co (min) = 80 mg; Cu (min) = 1000 mg; I (min) = 150 g; Mn (min) = 700 mg; Mg (min) = 8000 mg; Se (min) = 18 mg; S (min) = 22,000 mg; Zn (min) = 3600 mg (SalBov 90 LC, Walter Vale ME).

2.3. Continuous Culture Procedures

Rumen fluid was collected approximately 2 h after feeding from three rumen-cannulated male cattle (average BW = 450 kg). The donor cattle were fed a diet with a 60:40 concentrate-to-forage ratio, similar to that used in the experiments. The donors' diet consisted of corn silage (40%), soybean meal (13.5%), ground corn (45%), and minerals (1.5%) for at least 14 days

before rumen fluid collection for each incubation period. The rumen digesta was manually collected and strained through four layers of cheesecloth, placed in an insulated thermal container, and preheated until reaching a total volume of 15 L. After collection, the rumen fluid was pooled together and homogenized in a 5000 mL Erlenmeyer flask kept at 39 °C in a preheated water bath. The mixed rumen fluid was then transferred to each fermenter until it overflowed into the solid phase container (approximately 1297 mL per fermenter).

Fermenters were fed 55 g of dry matter per day, divided equally into two meals at 08:00 and 20:00 h. The fermenter contents were continuously stirred by a central propeller device at a rate of 350 rpm. A buffer solution [21] was infused continuously at 2.16 mL/min to achieve a dilution rate of 10% per hour. The effluent outflow was adjusted daily to ensure partitioning into 5% per hour of liquid effluent withdrawn by suction through a filter and 5% per hour of solid plus liquid withdrawn by overflow. Individual pH controllers (Accumet AP61, Fisher Scientific, Atlanta, GA, USA) were used to monitor the pH of each fermenter.

The liquid and solid effluents were individually collected in plastic containers. During the first 7 days of each period, the effluent containers were weighed once a day at 06:00, and the contents were discarded. Twenty-four hours before the first collection and during the 3-day (8, 9, and 10) sampling period, the liquid and solid effluent containers were refrigerated at 4 °C to minimize microbiological activity after removal from the vessel.

On days 8, 9, and 10, the liquid and solid digesta effluents from each fermenter were collected and mixed. A 500 mL sample was taken and stored at −20 °C for further chemical analysis. Additionally, two 10 mL subsamples were filtered through eight layers of cheesecloth, preserved with 0.2 mL of sulfuric acid (H₂SO₄; 50% concentration), and centrifuged at 1000× *g* for 15 min at 4 °C. The supernatant was then centrifuged again at 20,000× *g* for 30 min, and the resulting supernatant was stored for further ruminal analysis of ammonia nitrogen (N-NH₃) and short-chain fatty acids (SCFAs). The pH of the fermenter was measured with an Accumet AP61 pH meter (Fisher Scientific, Atlanta, GA, USA) at 01:00, 02:00, 04:00, 06:00, 08:00, 10:00, and 12:00 h.

On day 10, the entire fermenter content was filtered through four layers of cheesecloth. The remaining solid fraction from the filtration was washed with 200 mL of saline solution (0.9% NaCl, weight/volume) and centrifuged at 1000× *g* for 10 min at 5 °C. The supernatant was then centrifuged at 17,700× *g* for 23 min. After the second centrifugation, the supernatant was discarded, and the bacterial pellets were lyophilized and stored for further analysis of total purines, total nitrogen, and organic matter (OM) to estimate bacterial protein synthesis [22]. Apparent and true digestibilities were calculated according to Soder et al. [23] as follows (using CP as an example): CP apparently digested (%) = [(g of CP intake − g of effluent flow CP)/g of CP intake] × 100; CP truly digested (%) = [(g of CP intake − (g of effluent flow CP − g of microbial CP))/g of CP intake] × 100. In both equations, the effluent was corrected for the grams of buffer.

2.4. Chemical Analysis

All chemical analyses were performed in triplicates for each treatment during each period. The coefficients of variation were maintained below 5% to ensure consistency and reliability of the results. All ingredients were ground using a Willey mill (Model R-TE 648; Tecnal Equipamentos Científicos, Piracicaba, SP, Brazil) to achieve a 2 mm particle size. The samples of feed and effluent were analyzed for dry matter (DM, method 934.01), ether extract (EE, method 920.85), and ash (method 938.08) according to AOAC [24]. The crude protein (CP) content of the samples was determined using a Dumas therm combustion nitrogen analyzer (method 990.13) [25]. The OM content was calculated as the difference between the DM content and ash. For neutral detergent fiber (NDF), the samples were

analyzed, treated with thermostable α -amylase [26], and adapted for the Tecnal TE-149 fiber analyzer (Tecnal Equipamentos Científicos, Piracicaba, SP, Brazil). The ADF content was determined according to the methods of AOAC [25] using the Tecnal TE-149 fiber analyzer.

The ammoniacal nitrogen (N-NH₃) content was analyzed using the colorimetric method described by Chaney and Marbach [27]. The concentrations of SCFAs were determined by gas chromatography using a Varian gas chromatograph (model CP-3380; Varian, Inc., Walnut Creek, CA, USA) equipped with a flame ionization detector and an 80/120 Carbowax B-DA/4% Carbowax 20 M column, measuring 1.8 m in length with a 2 mm internal diameter. The nitrogen gas flow rate was 30 mL/min. The injector, detector, and column temperatures were set at 200 °C, 200 °C, and 175 °C, respectively. The total purine content of the bacterial pellets was determined using the method of Ushida et al. [28], as modified by Makkar and Becker [29]. The estimated microbial protein synthesis was calculated from the flow of purines, according to the equations proposed by Chen and Gomes [30].

2.5. Statistical Analysis

The results were analyzed using the MIXED procedure in SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA). Data were tested for normality using the Shapiro–Wilk test with the UNIVARIATE procedure. Data showing a non-normal distribution were transformed using either the logarithmic or square root method, whichever resulted in a more symmetric distribution. To investigate the effects of diet, each variable was tested separately and analyzed using the MIXED procedure according to the model $Y_{ijk} = \mu + T_i + P_j + \varepsilon_{ijk}$, where Y_{ijk} is the dependent variable, μ is the overall mean, T_i is the treatment effect ($i = 1$ to 5), P_j is the period effect ($j = 1$ to 5), and ε_{ijk} is the residual error. Data were analyzed using a 5 × 5 Latin square design (5 periods and 5 treatments) for both peanut meal and cottonseed meal experiments. For multiple comparisons, means were separated using Tukey's test at a 5% significance level. Significant effects were noted at $p \leq 0.05$, and trends were noted from $p > 0.05$ to $p \leq 0.10$.

3. Results

3.1. Cottonseed Meal

The true digestibility of DM and the apparent digestibility of CP differed among the processed meal groups (Table 3). The tannin treatment tended to have a lower true digestibility of DM compared to the control, autoclave, and oven treatments ($p = 0.09$). Additionally, tannin fermenters exhibited a lower apparent digestibility of CP compared to all other treatments ($p = 0.04$). No significant differences were observed for the other digestibility and fermentation parameters ($p > 0.20$).

The autoclave treatment exhibited lower flows of total N, non-ammonia N (NAN), and microbial N compared to other treatments (Table 4; $p < 0.01$). On the other hand, the oven treatment exhibited the highest flow of microbial nitrogen ($p < 0.01$), though this was not statistically different from the tannin treatment. Additionally, the tannin and microwave treatments exhibited the highest flow of dietary N and the lowest RDP-N supply ($p < 0.01$). The control treatment had a greater flow of NH₃-N compared to other treatments ($p < 0.01$). However, there were no significant differences in nitrogen utilization efficiency (NUE) and microbial efficiency among the cottonseed meal groups ($p = 0.95$).

Table 3. Effects of different processing techniques on the digestibility and fermentation of cottonseed meal diets in a dual-flow continuous culture system.

Item ¹	Cottonseed Meal					SEM	p-Value
	Control	Autoclave	Oven	Microwave	Tannin		
n	5	5	5	5	5		
True digestibility, g/kg							
Dry matter	482 ^d	490 ^d	520 ^d	405 ^{de}	331 ^e	48.6	0.09
Organic matter	613	613	654	621	523	40.0	0.25
Crude protein	782	800	894	819	748	47.3	0.30
Apparent digestibility, g/kg							
Dry matter	316	291	350	268	237	74.2	0.82
Organic matter	470	450	506	457	417	54.1	0.85
Crude protein	608 ^a	607 ^a	659 ^a	565 ^a	373 ^b	56.2	0.04
Neutral detergent fiber	799	768	855	805	736	59.6	0.70
pH	6.90	6.89	6.92	6.92	7.07	0.09	0.45
Total SCFAs, mM	61.7	60.0	57.4	59.7	59.2	4.34	0.97
SCFA profile, mol/100 mol							
Acetate	65.4	65.7	63.1	66.7	65.3	1.84	0.89
Propionate	23.7	23.4	26.3	24.9	25.3	1.09	0.32
Butyrate	10.9	11.0	10.6	8.45	9.46	1.42	0.68
Acetate: propionate	2.80	2.84	2.43	2.69	2.59	0.185	0.62
NH ₃ -N, mg/dL	10.7	10.5	8.47	11.9	9.54	1.62	0.79

^{a,b} Means with different superscripts in the same row are different ($p < 0.05$). ^{d,e} Means with different superscripts in the same row are different ($0.05 < p < 0.10$). ¹ SCFAs = short-chain fatty acids; SEM = standard error of the mean.

Table 4. Effects of different processing techniques on the nitrogen metabolism of cottonseed meal diets in a dual-flow continuous culture system.

Item ¹	Cottonseed Meal					SEM	p-Value
	Control	Autoclave	Oven	Microwave	Tannin		
n	5	5	5	5	5		
Nitrogen flow, g/d							
Total N	0.88 ^a	0.41 ^b	0.96 ^a	0.97 ^a	1.06 ^a	0.084	<0.01
NH ₃ -N	0.04 ^a	0.02 ^b	0.02 ^b	0.02 ^b	0.02 ^b	0.005	<0.01
NAN	0.84 ^a	0.37 ^b	0.94 ^a	0.95 ^a	1.04 ^a	0.082	<0.01
Microbial N	0.56 ^b	0.26 ^c	0.82 ^a	0.58 ^b	0.68 ^{ab}	0.077	<0.01
Dietary N	0.28 ^b	0.11 ^c	0.13 ^{bc}	0.37 ^a	0.36 ^a	0.053	<0.01
RDP-N supply	1.16 ^{bc}	1.33 ^a	1.31 ^{ab}	1.07 ^c	1.08 ^c	0.051	<0.01
NUE ²	32.3	38.4	41.5	41.6	43.9	8.16	0.95
Microbial efficiency ³	16.3	17.8	18.9	19.2	21.3	4.31	0.95

^{a,b,c} Means with different superscripts in the same row are different ($p < 0.05$). ¹ NH₃-N = ammonia nitrogen; NAN = non-ammonia nitrogen; RDP = rumen-degradable protein; NUE = nitrogen utilization efficiency; SEM = standard error of the mean. ² NUE = g of microbial N/g of available N. ³ Microbial efficiency = g of microbial N/kg of organic matter truly digested.

3.2. Peanut Meal

The autoclave and tannin treatments exhibited the highest acetate concentrations ($p = 0.03$; Table 5), though this was not statistically different from the control treatment. Furthermore, tannin had the lowest apparent digestibility of CP ($p < 0.01$). No significant differences were observed for the other digestibility and fermentation parameters ($p > 0.20$).

Regarding nitrogen metabolism (Table 6), the tannin treatment exhibited a lower RDP-N supply compared to all other treatments ($p = 0.02$). Similar results for all other nitrogen metabolism parameters were observed across all treatments in this study ($p > 0.20$).

Table 5. Effects of different processing techniques on the digestibility and fermentation of peanut meal diets in a dual-flow continuous culture system.

Item ¹	Peanut Meal					SEM	<i>p</i> -Value
	Control	Autoclave	Oven	Microwave	Tannin		
n	5	5	5	5	5		
True digestibility, g/kg							
Dry matter	432	461	523	477	500	43.6	0.65
Organic matter	590	619	560	603	615	46.6	0.88
Crude protein	908	811	875	844	722	49.9	0.21
Apparent digestibility, g/kg							
Dry matter	244	292	323	287	215	39.2	0.45
Organic matter	428	443	407	440	388	43.1	0.91
Crude protein	788 ^a	688 ^b	762 ^a	752 ^{ab}	489 ^c	27.0	<0.01
Neutral detergent fiber	832	817	826	815	768	51.8	0.94
pH	6.73	6.73	6.71	6.66	6.73	0.09	0.61
Total SCFAs, mM	56.8	59.6	61.8	64.7	58.9	4.90	0.83
SCFA profile, mol/100 mol							
Acetate	66.9 ^{ab}	67.1 ^a	64.9 ^b	65.4 ^b	67.4 ^a	0.73	0.03
Propionate	24.4	24.1	25.0	24.4	23.8	0.78	0.93
Butyrate	9.10	8.82	10.1	10.3	8.79	0.59	0.36
Acetate: propionate	2.74	2.80	2.61	2.77	2.85	0.09	0.43
NH ₃ -N, mg/dL	18.3	15.1	16.8	19.3	12.4	3.09	0.69

^{a,b,c} Means with different superscripts in the same row are different ($p < 0.05$). ¹ SCFAs = short-chain fatty acids; SEM = standard error of the mean.

Table 6. Effects of different processing techniques on the nitrogen metabolism of peanut meal diets in a dual-flow continuous culture system.

Item ¹	Peanut Meal					SEM	<i>p</i> -Value
	Control	Autoclave	Oven	Microwave	Tannin		
n	5	5	5	5	5		
Nitrogen flow, g/d							
Total N	0.54	0.87	0.76	0.85	0.72	0.129	0.43
NH ₃ -N	0.02	0.03	0.02	0.02	0.03	0.006	0.29
NAN	0.80	0.84	0.73	0.82	0.93	0.076	0.53
Microbial N	0.66	0.65	0.55	0.59	0.56	0.075	0.81
Dietary N	0.14	0.28	0.17	0.24	0.37	0.079	0.40
RDP-N supply	1.53 ^a	1.36 ^a	1.49 ^a	1.42 ^a	1.07 ^b	0.079	0.02
NUE ²	39.4	39.4	33.2	35.4	39.3	4.53	0.78
Microbial efficiency ³	22.4	18.0	19.1	19.0	18.4	2.50	0.76

^{a,b} Means with different superscripts in the same row are different ($p < 0.05$). ¹ NH₃-N = ammonia nitrogen; NAN = non-ammonia nitrogen; RDP = rumen-degradable protein; NUE = nitrogen utilization efficiency; SEM = standard error of the mean. ² NUE = g of microbial N/g of available N. ³ Microbial efficiency = g of microbial N/kg of organic matter truly digested.

4. Discussion

4.1. Cottonseed Meal

Tannins are phenolic compounds found in certain plants that can be utilized in ruminant nutrition to decrease protein digestibility [31]. They achieve this by modulating ruminal fermentation, with a significant impact on protein [32]. Tannins primarily interact with dietary amino acids, forming hydrogen bonds and reducing their ruminal degradation [33]. Thus, as expected, we observed that the addition of tannin reduced protein availability for microorganisms due to its strong complexation ability, thereby decreasing the apparent digestibility of CP. A similar trend was observed in true DM digestibility, likely related to the decrease in apparent CP digestibility. However, it is important to note that this reduction in apparent digestibility was not significant enough to affect the true digestibility of CP. These results are interesting because they suggest that despite the effects on protein protection, the tannin treatment was capable of providing an adequate supply of nutrients for microorganism growth. This is supported by the results for microbial nitrogen (N)

flow, where tannin treatment showed similar microbial N flow to the control, microwave, and oven treatments and greater flow than the autoclave treatment. Other studies have also observed a reduction in ruminal protein degradation with increasing tannin levels in ruminant diets [33–35]. Given the absence of differences in digestibility and fermentation parameters among the various processing techniques for cottonseed meal diets, these results indicate that the evaluated methods did not impact nutrient fermentation in the rumen, maintaining stable parameters comparable to the control. The lack of significant differences in nitrogen utilization efficiency (NUE) and microbial efficiency suggests that the processing methods evaluated did not substantially affect the conversion of nitrogen into microbial protein, a critical source of amino acids for ruminants [22]. This stability may reflect the maintenance of an adequate ruminal environment despite changes in dietary nitrogen availability [14]. However, it also highlights the limitations of these processing methods in improving these metrics, emphasizing the need to explore combinations of techniques that maximize NUE in high-performance systems.

Heating feed can alter the availability of certain nutrients, such as protein, due to complexation with other compounds, like carbohydrates [36,37]. Consequently, in terms of nitrogen metabolism variables, the lower values for total nitrogen (N), ammonia nitrogen (N-NH_3), microbial nitrogen (microbial N), and dietary nitrogen (dietary N) suggest that wet heating may significantly impact the nutrient chemical profiles [38]. Another interesting result observed in this study was the highest N-NH_3 level in the control group compared to other processing methods. During fermentation, a significant portion of ingested nitrogen (protein) is converted into compounds that include carbon chains and N-NH_3 [39]. Thus, these results suggest that processed treatments may enhance the conversion of N-NH_3 into microbial protein, as evidenced by the greater microbial N flow in the oven and tannin treatments. Conversely, the autoclave treatment showed the lowest microbial N flow, which is undesirable and could be due to overprotection from Maillard reaction products or specific interactions between the processing method and the meal [36,40,41].

We hypothesized that processing methods could interfere with protein metabolism in the rumen, protecting this fraction of the feed and altering its ruminal availability, thereby reducing the supply of RDP-N [8–10]. Our results confirmed this hypothesis for the microwave and tannin treatments, indicating the potential of these two processing techniques to increase the RUP of cottonseed meal. From a biological perspective, the increase in dietary nitrogen for these treatments reinforces the observed reduction in RDP-N. The mechanisms of protein protection by tannins were discussed earlier. On the other hand, the effectiveness of microwave heating can be attributed to its potent and uniform heating, which differs from other techniques [42–44]. Microwave heating effectively modifies protein complexes, reducing their availability at the ruminal level while increasing it at the intestinal level, thus improving nutrient utilization efficiency [45]. Additionally, the treatment with xylose can enhance the complexation of proteins with sugar induced by heat treatments [46–48]. Previous studies have demonstrated the effectiveness of microwave treatment alone [42,49,50] and in combination with xylose [51] to increase RUP levels in different feeds.

Therefore, our results suggest that both microwave and tannin processing methods have the potential to increase the RUP levels of cottonseed meal. This processed feed could be used in ruminant diets to enhance dietary protein flow to post-rumen compartments without impairing overall ruminal fermentation.

4.2. Peanut Meal

Peanut meal offers a favorable nutrient profile, rich in amino acids, but is characterized by high ruminal protein degradation [52–54]. To address this issue, various processing

methods are being explored, as evaluated in this study. Similar to cottonseed meal diets, the application of tannin to peanut meal resulted in lower protein digestibility compared to other treatments. The reduced CP digestibility in tannin-treated peanut meal is due to the formation of strong tannin–protein complexes that resist ruminal degradation [55,56]. Tannins can affect nutrient interaction within the animal’s digestive tract, reducing their availability and absorption efficiency [55]. Despite this reduction, the treatment maintained microbial efficiency, suggesting adequate nutrient availability for ruminal microorganisms. However, high tannin levels could impair total digestibility, emphasizing the need for correct dosage [56]. Additionally, autoclaving also showed the potential to decrease protein digestibility compared to the control. However, this change was not observed in the tannin treatment. Moreover, the reduction in apparent digestibility without changes in other fermentation parameters suggests that these processing methods could be effective for protecting protein from rumen fermentation.

Regarding nitrogen flow variables, significant differences were observed only in RDP-N supply, with tannin processing showing lower values compared to all other treatments. Tannins are known for their ability to complex with nutrients, reducing feed degradability without compromising intestinal digestion [56–58]. This reduction in nitrogen supply from RDP can be linked to the previously mentioned apparent protein digestibility. A decrease in protein degradation due to tannin was noted, which reduced the protein available in the rumen. Additionally, despite the lack of statistically significant differences, the tannin treatment exhibited a dietary N flow 2.64-fold higher than the treatment with untreated peanut meal. Moreover, tannin processing demonstrated bacterial efficiency similar to the control, which does not involve any protein protection processing, indicating that tannin was effective in maintaining ruminal microbial activity without hindering its development. In our study, we expected that all processing methods would increase RUP and consequently reduce RDP-N flow. However, the inefficacy of all heat treatments (autoclave, oven, and microwave) in enhancing dietary nitrogen, compared to the control, was unexpected. This could be attributed to overheating, which may reduce ruminal nitrogen metabolism or interactions among ingredients. Nonetheless, our findings suggest that the tannin processing method can effectively elevate RUP levels in cottonseed meal. This processed feed can be integrated into ruminant diets to improve intestinal protein digestion without negatively impacting overall ruminal fermentation. These results could enhance protein utilization in beef cattle, leading to improved outcomes. This is especially important for high-yielding cattle, which have greater RUP requirements, enabling them to achieve optimal productivity. However, further *in vivo* studies are needed to confirm these results.

5. Conclusions

Our results indicate that tannin (4 g tannin inclusion per kg of peanut meal) and microwave (feed treated with 2% xylose and heated in a microwave oven for 6 min) processing were the most effective methods for reducing the protein fraction available in the rumen for cottonseed meal. For peanut meal, the findings also highlight the potential of tannin (4% tannin inclusion) as a strategy to increase the RUP in ruminant diets without negatively affecting ruminal fermentation. The controlled application of microwave heat to cottonseed meal and the strategic inclusion of tannins in both cottonseed and peanut meals can enhance nutrient utilization, thereby improving feed efficiency and reducing production costs. However, some variables exhibited trends or biologically unexplained results, underscoring the need for further research to gain a deeper understanding of these effects.

Author Contributions: Conceptualization, P.D.B.B. and E.M.P.; methodology, K.E.L., M.J.S., S.B.D., J.G., B.R.A., E.M., and E.M.P.; validation, T.H.S., P.D.B.B., E.M., and E.M.P.; formal analysis, T.H.S., P.D.B.B., and E.M.P.; investigation, K.E.L., P.D.B.B., E.M., and E.M.P.; resources, R.H.B., P.D.B.B., and E.M.P.; data curation, K.E.L., T.H.S., P.D.B.B., E.M., and E.M.P.; writing—original draft preparation, K.E.L., T.H.S., P.D.B.B., and E.M.P.; writing—review and editing, K.E.L., M.J.S., S.B.D., J.G., B.R.A., E.M., T.H.S., R.H.B., P.D.B.B., and E.M.P.; visualization, K.E.L., M.J.S., S.B.D., J.G., B.R.A., E.M., T.H.S., R.H.B., P.D.B.B., and E.M.P.; supervision, E.M., R.H.B., P.D.B.B., and E.M.P.; project administration, R.H.B., P.D.B.B., and E.M.P.; funding acquisition, R.H.B., P.D.B.B., and E.M.P. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully acknowledge the São Paulo Research Foundation (FAPESP) for funding the project (FAPESP; Grant # 2017/50339–5; 2018/19743–7; 2019/17243–0; 2019/22626–2; 2021/09562–8). They also thank the members of the Laboratory of Nutrition and Ruminant Fermentation of the Beef Cattle Research Center of Instituto de Zootecnia for their assistance with sample collection and laboratory analyses. The authors also acknowledge the funding received from the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC, public notice number 48/2022, grant 2023TR000535).

Institutional Review Board Statement: This study was carried out in strict accordance with the recommendations of the Animal Use Ethics Committee of the Instituto de Zootecnia. Animal care and the handling protocol were approved by this committee (Protocol Number 249-19).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: The authors gratefully acknowledge the farm crew at the Experimental Station (Instituto de Zootecnia) for animal feeding and care.

Conflicts of Interest: The authors declare no conflicts of interest.

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