FEDERAL UNIVERSITY OF BAHIA
GRADUATE PROGRAM IN ANIMAL SCIENCE

SLOW-RELEASE UREA IN THE LAMB RUMEN BY MICROSPHERES VEGETABLE FAT

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SUMMARY

1. GENERAL INTRODUCTION

The evolution of ruminants has enabled these animals to utilize the carbohydrates present in plant cell walls and non-protein nitrogen compounds. This has only been possible due to the symbiotic relationship between these animals and the diverse and numerous microbial populations in their rumen-reticulum. The degradation of carbohydrates and proteins primarily generates volatile fatty acids, NH^3, microbial protein, and gases (CO^2 and CH^4), most of which, except for the gases, are used by the animal to meet its energy and amino acid requirements.

Urea is considered the product of nitrogen metabolism, resulting from the ammonia detoxification in most mammalian species. Ruminants have evolutionarily developed the ability to reuse urea in the gastrointestinal tract, especially in compartments with a large microbial population, using this recovery mechanism to maintain nitrogen balance in their bodies (NICHOLS *et al*., 2022). Thus, the rumen is the primary site where urea is recycled and can be reused as a substrate for microbial protein synthesis, contributing to the flow of digestible N (LAPIERRE; LOBLEY, 2001).

Protein solubility has been associated with increased ruminal degradation, which is related to the accumulation of ammonia in the rumen fluid. Ammonia is the primary nitrogen source for bacteria that degrade fibrous carbohydrates. Under dietary conditions limiting in this nutrient, these bacteria exhibit slower growth due to higher maintenance costs, partly represented by the use of energy-consuming ammonia assimilation systems (ATP), such as the glutamine synthetase/glutamate synthase complex, or the existence of futile proton cycles (NOCEK AND RUSSELL, 1988; NOLAN, 1993; RUSSELL AND STROBEL, 1993; BROCK *et al*., 1994).

Nitrogen recycling can be an essential source of this nutrient in situations of dietary scarcity. When dietary nitrogen levels are low, 70% of ingested nitrogen can be recycled and conserved by the rumen-hepatic cycle (50 g CP kg^{-1} DM). However, when nitrogen content is high (200 g CP kg^{-1} DM), nutrient recycling decreases dramatically to approximately 11% (NRC, 1989), which can result in considerable losses and reduced dietary nitrogen utilization efficiency (VAN SOEST, 1994).

One strategy to increase the efficiency of urea use in ruminant feeding is its partial protection through techniques such as encapsulation, microencapsulation, and emulsion, using polymers or lipid sources. These methods promote the slow release of urea in the

rumen, preventing its toxicity (CARVALHO *et al*., 2019b; MEDEIROS *et al*., 2019). The critical characteristics desired in a product for microencapsulating urea for slow release are that it should be inert in the rumen and hydrophobic. In this context, vegetable fat (VF), typically used in culinary blends, emerges as a promising option for urea protection.

VF offers advantages such as ease of acquisition and processing, as well as desirable physicochemical characteristics to act as an encapsulant. Its composition, with a higher concentration of saturated fatty acids and a lower concentration of polyunsaturated fatty acids due to the hydrogenation process, may reduce VF toxicity to rumen microorganisms and make it inert in the rumen environment. However, evaluating the effect of the fatty acids present in VF in the ruminant diet is crucial. This consideration arises from the fact that fatty acids ingested by ruminants can undergo biohydrogenation in the rumen, altering the fatty acid profile. This alteration can potentially reduce trans-fatty acids that would otherwise be deposited in meat and consumed by humans.

Considering the encapsulation system for slow urea release in the rumen of lambs and all the characteristics and potential of VF as a wall material, we hypothesize that VF can be used as a coating material to protect urea from rumen microorganisms, allowing gradual release in the rumen environment, improving nutrient synchronization and degradation, reducing the presence of soybean meal in the diet, and the risk of ammonia intoxication.

Therefore, the objective of this study was to obtain, characterize, and define the ideal ratio of vegetable fat/urea for slow urea release; understand the effects of its inclusion in sheep diets compared to conventional urea use on nutrient metabolism, rumen parameters, nitrogen metabolism, carcass characteristics, meat quality, and fatty acid profile of lamb meat.

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CHAPTER 1

Slow-releasing urea in the rumen by microspheres lipidic of vegetable fat: production, characterization, and nitrogen metabolism in lambs

SLOW-RELEASING UREA IN THE RUMEN BY MICROSPHERES LIPIDIC OF VEGETABLE FAT: PRODUCTION, CHARACTERIZATION, AND NITROGEN METABOLISM IN LAMBS

ABSTRACT

This study characterized and defined the optimal ratio of low-trans vegetable fat (LTVF) and urea that enables protection of urea for slow-release in rumen by evaluating the physicochemical properties of the material, and the effect of inclusion of levels of the best ratio compared to conventional urea in sheep diets. In the first experiment, three LTVF ratios of 30:70 (SRU30), 40:60 (SRU40) and 50:50 (SRU50). All formulations showed microencapsulation yields above 80%, but the proportion SRU40 showed higher microencapsulation efficiency (85.5%), retaining the highest percentage of urea with 115% crude protein (CP) and higher thermal stability ($P < 0.05$). In the second experiment, the SRU40, just named SRU was included in the diets of lambs, evaluating five treatments: a control treatment with 0.5% free urea (U0.5) in DM and four treatments with SRU40 levels added in the diets at the proportions of 1.25% (SRU1.25), 2.0% (SRU2) and 3.0% (SRU3) of total diet DM. To evaluate the N metabolism, 24 uncastrated Santa Inês lambs with a mean age of six months and mean body weight of 16 ± 2.2 kg were distributed in a randomized block design with two blocks, four treatments and six replications. To evaluate rumen parameters, four Santa Ines sheep with approximately 40 ± 0.5 kg body weight, fistulated and cannulated in the rumen, were distributed in a double 4 × 4 Latin square. The different forms of urea offered (free and protected) and levels of SRU inclusion did not change the intake of DM and N. SRU linearly reduced ($P < 0.05$) N-urinary excretion, impacting on the linear increase of N retention and microbial protein. The inclusion of SRU linearly reduced ($P < 0.05$) the rumen pH at 1 h, 4 h, and 6 h after feeding, but not at 2 h ($P = 0.25$) after feeding. The inclusion of SRU linearly increased the concentration of BUN and tended to linearly increase the concentration of total proteins and AST ($P < 0.05$). SRU1.25 promoted lower BUN concentration ($P < 0.05$) compared to U0.5, and SRU1.25 diets tended ($P = 0.059$) to have lower serum magnesium in sheep compared to U0.5. BUN in lambs linearly decreased in relation to offer time and treatments, with the highest BUN concentrations for U0.5 at 0, 4 and 6 h after feeding ($P < 0.05$). SRU diets promoted BUN peak after 2 h of feeding, with the same concentration compared U0.5 ($P = 0.117$). Rumen pH decreased linearly 1 h after feeding as well as 4

h and 6 h ($P < 0.05$), however, it did not vary at 2 h ($P = 0.246$). There was a linear increase at 1, 2, 4 and 6 h after the first hour pos-feeding ($P < 0.05$). Low-trans vegetable fat was efficient in encapsulating urea, especially the formulation in the proportion of 40% urea and 60% vegetable fat (SRU40). SRU40 is recommended in sheep's diets with up to 3% as DM, replacing soybean meal, as it improves N metabolism of animals.

KEYWORDS: Hydrogenated fat; NH3-N; Nitrogen metabolism; Thermogravimetry; Ruminant.

1. INTRODUCTION

Non-protein nitrogen (NPN) sources are commonly used to feed ruminant animals, primarily strategically reducing feed costs by replacing part of the soybean meal and providing a readily available nitrogen source (N) to rumen microorganisms. One such source is urea, which once ingested is rapidly hydrolyzed in the rumen within 30 minutes to 2 hours (Rekib and Sadhu, 1968). However, carbohydrate degradation in the rumen and subsequent microbial growth are much slower processes. Therefore, greater synchrony of these processes may improve the efficiency of incorporation of NPN into microbial protein and improve overall N use efficiency (TAYLOR-EDWARDS *et al*., 2009).

Several factors, including the availability of fermentable carbohydrates in the rumen, diet composition, rate of feed passage through the digestive tract, and the efficiency of nitrogen utilization by rumen bacteria regulate the metabolism of ammonia nitrogen (NH^3-N) in ruminant animals. When ingested at high levels, urea is rapidly hydrolyzed to NH^3-N in the rumen and can be toxic to ruminants. The factors that can contribute to NH^3-N toxicity include rumen pH, dietary fiber level, and animal adaptation (PUNIYA *et al*., 2015).

One of the strategies used to increase the use of urea in ruminant feeding is its protection by polymers or lipid sources through different methods (encapsulation, microencapsulation, and emulsion), thus promoting its slow release in the rumen. Some studies have been developing new methods of protection, such as the study of Melo *et al*. (2021), who used polymeric microparticles of calcium pectinate to encapsulate urea and managed to improve the utilization of N by sheep and reduce the risk of intoxication. Other authors have used waxes to protect urea by microencapsulation due to their high melting point and stability in the rumen, such as carnauba wax (DE MEDEIROS *et al*., 2019) and beeswax (CARVALHO *et al*., 2019). Although these materials are efficient in protecting urea in the rumen environment, they are difficult to process and to obtain. It is the case of waxes, which depend on harvest, which makes them expensive and difficult to use on a large scale. Thus, it is necessary to search for other materials that are more accessible for the slow release of urea in the rumen.

The oil hydrogenation process involves a chemical procedure in which hydrogen atoms are introduced to unsaturated fatty acids (UFA), increasing the concentration of saturated fatty acids (SFA) in the oil (COENEN, 1976). Depending on the extent of

hydrogenation, this can permanently alter the oil's state, transitioning it from a liquid to a semi-solid or even solid form at room temperature. This modification enables the spreadability of the solidified oil (WONGJAIKHAM *et al*., 2022). The primary objective of this procedure is to elevate the oil's melting point, enhance its oxidation resistance, and extend its shelf life (DANIELS *et al*., 2006). This technique has found widespread application in manufacturing margarine and vegetable shortening. Margarine, recognized for its cost-effectiveness and extended shelf life, has been widely adopted as a butter substitute. It is famously characterized as a water-in-oil emulsion (LI *et al*., 2018) and is frequently incorporated as an ingredient in various bakery items, including cakes, bread loaves, and cookies. However, the production of margarine through the partial hydrogenation of vegetable oils is frequently associated with the generation of LTVF, which has physical and chemical characteristics with affinity for use as an encapsulating material, providing a slow release of water-soluble materials such as urea (DE MEDEIROS *et al*., 2019, NETTO *et al*., 2021).

The encapsulating (protection) agent selection depends on the method used to form the capsules, the product's application type, and how it will act. The LTVF characteristics may be attractive when using this material as a wall in developing a product to protect urea. In addition, LTVF has in its composition a higher concentration of SFA and a lower concentration of polyunsaturated fatty acids (PUFA), due to the hydrogenation processing, which may reduce its toxicity to rumen microorganisms and may be inert in the rumen environment. Since LTVF is an easily acquired and processed material, besides its adequate physicochemical characteristics for an encapsulant, which are the stability and the composition of FA, we hypothesized that it could be used as a coating material for the protection of urea from rumen microorganisms, allowing the gradual release into the rumen environment, improving the synchronization and degradation of nutrients, and reducing the of soybean meal in the diet and the risk of ammonia intoxication.

Therefore, the objective of this study was to obtain, characterize, and define the ideal proportion of low-trans vegetable fat and urea that enables the protection of urea for slow release by evaluating the physicochemical properties of the material and to evaluate the effect of the diet formulation compared to conventional urea on rumen parameters and nitrogen metabolism in sheep.

2. MATERIAL AND METHODS

Two separate experiments were performed for the evaluation of urea protected by low-trans vegetable fat (LTVF). In the first experiment, different concentrations of urea and LTVF were tested to produce the protected slow-release urea (SRU). In the second experiment, after determining the ideal ratio to obtain the best urea protection system, the material was included in the diet of sheep to evaluate the behavior of the encapsulated in the rumen and its effects on nitrogen metabolism.

1st experiment: Obtaining and characterizing slow-release urea protected by low-trans vegetable fat.

2.1. EXPERIMENTAL DESIGN AND OBTENTION OF SLOW-RELEASE UREA (SRU) SYSTEMS

To determine the best ratio between U (core) and LTVF (encapsulant), three rumen slow-release urea (SRU) formulations were tested with different ratios (mass/mass) between core and encapsulant. The tested ratios of U:LTVF were: 30:70 (SRU30), 40:60 (SRU40) and 50:50 (SRU50). The LTVF (Cukin® vegetable fat, Bunge Alimentos S.A., Brazil) had in its composition hydrogenated vegetable oils (mainly soybean oil), antioxidants (TBHQ and citric acid) and dimethylpolysiloxane defoamer and was characterized with a minimum smoke point of 225° (RIBEIRO *et al*., 2009). SRU was then produced from the Fusion-emulsification technique of Medeiros *et al*. (2019), using soy lecithin as the emulsifying agent at a ratio of 1% mass of LTVF.

LTVF was weighed on an analytical scale and added to a beaker with a surfactant (40% soy lecithin) in the proportion of 1% of the vegetable fat mass. LTVF and lecithin were kept in a thermostatic bath at a temperature of 60°C. In another beaker, the urea was dissolved in distilled water to form a 50% (w/w) solution that had a pH of 9.2. The solution was kept in the thermostatic bath to facilitate the dissolution of urea and to equalize the temperature with LTVF.

After stabilizing the temperature of the materials, the urea solution was gradually added to the beaker containing LTVF and soy lecithin while mixing with a dispersers homogenizer crushing (T25 digital Ultra-Turrax®, Ika, USA). Finally, the emulsion was transferred to plastic containers and kept in a forced air circulation oven at a constant temperature of 55 °C for 24 hours for dehydration. After drying the material was removed

from the oven and when it reached the room temperature it was stored in a refrigerator at 2 ºC for further analysis and use.

Thus, a completely randomized experiment was designed with five treatments for the first experimental stage with three types of SRU microparticles prepared: SRU30 = low-trans vegetable fat (LTVF) microparticle containing urea (U) in the proportion 30:70; SRU40 = low-trans vegetable fat (LTVF) microparticle containing urea (U) in the proportion 40:60; and SRU50 = low-trans vegetable fat (LTVF) microparticle containing urea (U) in the proportion 50:50; plus free U and the LTVF used in the SRU preparation as controls for comparisons.

2.2. CHARACTERIZATION AND EVALUATION OF SLOW-RELEASE UREA (SRU) SYSTEMS

The microencapsulation yield (MY) was calculated based on the mass of LTVF, soy lecithin and 50% urea solution used initially, and the final mass after the drying process using the following equation: $MY = (M_{final}/M_{initial}) \times 100$; where M_{final} is the mass of the emulsion after drying; and $M_{initial}$ is the sum of the masses of vegetable fat, soy lecithin and 50% urea solution.

The microencapsulation efficiency (ME) expresses the ability of LTVF to retain urea in the system and was calculated based on the amount of nitrogen inserted in the form of urea into the system and the amount that remained after processing through the equation: $ME = (U_{retained}/U_{inserted}) \times 100$; where $U_{retained}$ is the urea content retained after processing; $U_{inserted}$ is the urea content inserted into the system.

The thermogravimetric curves were obtained in a thermogravimetric analyzer model TGA-50 Shimadzu, under an inert atmosphere (Argon), at a flow rate of 50 mL/min, heating rate of 10 °C/min, over a temperature range of 30 to 600 °C, using an alumina crucible containing an average of 6.0 mg of sample, as a function of temperature and time simultaneously. The plotting of TG and DTG curves and data analysis were performed in the OriginPro 8 software, in which the T_{onset} obtained from the TG curve was considered as a parameter to assess the initial degradation temperature, obtained by the tangent of the intersection between the upper horizontal baseline and the line drawn on the slope of the thermal event evaluated, being called extrapolated onset or mathematical onset of thermal degradation, as well as T_{max}, extracted from the DTG curve.

DSC curves were obtained in a differential scanning calorimeter model DSC-60, Shimadzu, under an inert atmosphere (Argon), in a flow of 50 mL/min, heating rate of 10 °C/min, over a temperature range of 30 to 300 °C, using a platinum crucible containing around 2.5 mg of sample. The plotting of the DSC curves and data analysis were performed in the OriginPro 8 software, where the peak temperature of the events was considered.

2nd Experiment: Animals, diets, and nitrogen (N) metabolism

2.3. ANIMAL MANAGEMENT, DIETS, EXPERIMENTAL DESIGN, DIGESTIBILITY, AND N BALANCE

For the 2nd experiment, after obtaining the results of the characterization of SRU, the proportion of 40% urea and 60% vegetable fat (SRU_{40}) was chosen to be tested for inclusion in the sheep diet. In the execution of all animal experiments, all management practices were performed only after approval (Protocol number 58/2021) and in strict concordance with the recommendations of the Ethics Committee on Animal Use (CEUA) of the Federal University of Campina Grande, Paraíba, Brazil.

Twenty-four uncastrated Santa Inês lambs, with a mean age of six months and mean body weight of 16 ± 2.2 kg, were distributed in a randomized block design, using the initial weight as a criterion for the formation of two blocks, with four treatments and six replications. The control treatment had 0.5% free urea (U0.5) in DM and the other treatments were composed of SRU40 added to the animals' diets in the proportions of 1.25% (SRU1.25); 2.0% (SRU2) and 3.0% (SRU3) of total diet DM, corresponding to 0.5, 0.8 and 1.2% of free urea in the diet, respectively.

The lambs were weighed before the beginning of the experiment fasting on solids, then were identified, vaccinated against clostridium disease (Biovet Resguard Multi®, São Paulo, Brazil), orally dewormed with 5% Levamisole hydrochloride (Ripercol® L, São Paulo, Brazil) and supplemented with vitamin mix (A, D and E). The animals were individually housed in metabolic cages equipped with drinking fountains, feeders, and compartments for collecting individual feces and urine. The experiment lasted for 28 days, with an initial 21-day period of animal adaptation to the environment, management practices, and diets, followed by a 7-day period for total collection of feces and urine samples. Samples of the diets and leftovers were collected daily and pooled into a composite sample for each animal, and frozen at -20 ºC for further analysis.

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

Item (g/kg DM)	Ground corn silage[1]	Free urea	SRU_{40}[2]	Soybean meal	Ground corn	Tifton–85 hay
Dry matter (g/kg as fed)	670	980	981	916	899	872
Crude ash	18.1	2.10	0.84	80.7	14.5	81.2
Crude protein	97.1	2784	1147	402	87.9	88.3
Ether extract	39.4	-	595	15.5	72.9	11.0
$_{ap}$Neutral detergent fiber[3]	128	-	-	157	115	729
Non-fiber carbohydrates	718	-	-	345	710	91.3
Cellulose	105	-	-	89.2	75.7	321
Hemicellulose	19.4	-	-	59.6	31.0	355
Acid detergent lignin	3.22	-	-	8.23	8.32	52.5

[1]Hydrated with cactus pear mucilage.
[2]SRU_{40} = slow-release urea produced from 60% lipid matrix of low-*trans* vegetal fat (LTVF) and 40% urea (U);
[3]$_{ap}$NDF, Neutral detergent fiber tested with heat stable amylase and corrected for ash and nitrogen compounds.

The diets were formulated to meet the requirements of growing male sheep for an average daily gain of 200 g/day according to the NRC (2007). Diets were formulated at a 30:70 roughage:concentrate ratio. The source of roughage was Tifton-85 hay (Cynodon sp) and the concentrate was composed of ground corn, corn grain silage hydrated by cactus mucilage (HCS), soybean meal, mineral salt, and free urea or SRU40 (Table 1). As a source of soluble carbohydrate and to enhance urea utilization in the rumen, corn grain silage hydrated by cactus pear mucilage (Opuntia stricta Haw.) was added and mixed at a corn grain: mucilage ratio of 75:25%. Free U and SRU40 were added to the diet based on total DM and mixed in a Y-mixer (Table 2). Pre-adaptation to urea was performed by providing urea gradually during the adaptation period. The diet was offered as a total mixed ration (TMR) in two equal parts to the lambs at 7am and 5pm and water was provided ad libitum.

N intake was obtained from the total DM offered in the diet and the total of each N in the leftovers expressed as grams per day (g/day). Total fecal samples were collected daily, then at the end of the collection period, a composite sample was made for each animal, which was identified and frozen, and pre-dried in a forced ventilation oven at 55

ºC and ground in a Willey type knife mill (Tecnal, Piracicaba, São Paulo, Brazil) with a 1-mm diameter sieve, for further analysis.

Table 2. Ingredient proportion and chemical composition of fiber of experimental lamb diets including slow-release urea (SRU) produced from the lipid matrix of low-trans vegetal fat.

Item	Free U (%DM)	SRU[1] (%DM)		
	0.5	1.25	2.0	3.0
Ingredients				
Tifton–85 hay	300	300	300	300
Ground corn	505	498	518	539
Ground corn silage[2]	20	20	20	20
Soybean meal	140	139.5	112	81
Free urea (U)	5.0	-	-	-
Slow-release urea (SRU40)[2]	-	12.5	20	30
Mineral mixture[3]	30	30	30	30
Chemical composition of diet (g/kg)				
Dry matter (g/kg as fed)	892	893	893	893
Crude ash	73.3	73.2	71.2	69.1
Crude protein	142	142	141	142
Ether extract	43.1	49.7	55.5	62.5
$_{ap}$Neutral detergent fiber[4]	301	300	298	295
Non-fiber carbohydrates	448	443	448	452
Cellulose	131	131	130	128
Hemicellulose	149	149	148	146
Acid detergent lignin	21,2	21,1	21,1	21,0
Neutral detergent insoluble protein[4]	312	310	312	314
Acid detergent insoluble protein[4]	194	192	196	199
Total digestible nutrients[5]	806	814	829	846

[1]SRU = slow-release urea produced from 60% lipid matrix of low-*trans* vegetal fat (LTVF) and 40% urea (U).
[2]Ground corn silage hydrated with cactus pear mucilage;
[3]Assurance levels (per kilogram of active elements): 120 g of calcium, 87 g of phosphorus, 147 g of sodium, 18 g of sulfur, 590 mg of copper, 40 mg of cobalt, 20

mg of chromium; 1,800 mg of iron, 80 mg of iodine; 1,300 mg of manganese, 15 mg of selenium; 3,800 mg of zinc, 300 mg of molybdenum; maximum 870 mg of fluoride

[4] $_{ap}$NDF, Neutral detergent fiber tested with heat stable amylase and corrected for ash and nitrogen compounds.

[5](g/kg CP)

Total daily urine collections were obtained in buckets containing sulfuric acid (H_2SO_4) at 0.036 N for 7 days, then the urine was homogenized, and a 10-mL aliquot from each animal was filtered, which were added and identified in flasks, and stored at -20º C for further analysis. The nitrogen (N) content of the collected urine and fecal samples were analyzed according to AOAC (method 981.10; 2015).

To determine the apparent digestibility coefficients (DC), the following equation was used: DC = [(g of nutrient or analytical fraction ingested - g of nutrient or analytical fraction excreted in feces)]/(g of nutrient or analytical fraction ingested) × 100. The total digestible nutrient intake (TDNI) was obtained from the difference between the intake and the recovered in the feces of each nutrient, on dry matter basis, according to the equation of Sniffen *et al*. (1992): TDNI (kg) = (digestible CP) + (2.25 × digestible EE) + (digestible NFC) + (digestible NDF). The contents of total digestible nutrients (TDN) were obtained from the following equation: TDN (g/kg) = (TDN intake / DM intake) × 100.

The nitrogen balance, expressed as daily amounts of nitrogen compounds, was calculated by the equation: N-retained (g/d) = N-intake (g/d) – N-fecal excretion (g/d) – N-urinary excretion (g/d). The variables referring to excretion and N-retained were further presented as a function of N-intake.

The excreted purine-derived compounds (PD) were determined by adding the daily excretion of allantoin and uric acid in urine, excluding the excretion of xanthine and hypoxanthine. This approach is adopted because of the high correlation between the sum of allantoin and uric acid with the concentration of nucleic acids in the rumen (TOPPS AND ELLIOTT, 1965; SANTOS *et al*., 2022). In small ruminant animals, xanthine and hypoxanthine values generally represent less than 1% of total PD. PD uptake was calculated based on the mathematical model described by Chen *et al*. (1990). Microbial nitrogen supply (MNS) was estimated according to Chen *et al*. (1992), and microbial protein synthesis (MPS) was calculated by multiplying MNS by 6.25. The efficiency of microbial protein synthesis was obtained by dividing the daily microbial protein production (in grams) by the daily TDN intake.

Blood was collected from all animals on the last day of the experimental period through jugular venipuncture using a vacuum system (Becton, Dickson and Co., São Paulo, SP, Brazil) immediately before feeding (0 h), and 2, 4 and 6 h after feeding. To facilitate collection and ensure animal welfare, the animals were trichotomized in the region of the external jugular vein and a nº 16 catheter (Medical supply®, São Paulo, Brazil) was inserted in each animal. The blood samples were temporarily kept at room temperature until the clot was formed and then centrifuged at 2,500 × g for five minutes in a Centrifuge 90-1 model (Coleman®, São Paulo, Brazil) to obtain the blood serum. The serum was stored at –20 °C in Eppendorf® tubes (Sigma-Aldrich, São Paulo, Brazil) until analysis. Blood urea nitrogen (BUN) was measured in commercial kits (Labtest@, Brazil) in an automated biochemical apparatus Cobas C111 (Roche, Germany).

2.4. RUMEN PARAMETERS

Four castrated adult Santa Inês sheep weighing 40 ± 0.5 kg, fistulated and cannulated in the rumen, were distributed in a double 4 × 4 Latin square (4 treatments and 4 periods), repeated in time. The animals were housed individually in metabolic cages, provided with drinking, and feeding troughs. The experiment lasted 84 days, divided into 4 periods of 21 days each. In each period, the first 14 days were for adaptation of the animals to the diets, and 7 days for collection. The diets were the same as in the previous experiments (animals in metabolic cages - Table 2) and followed the same pattern of mixing, feeding, and refusals collection.

Rumen fluid samples (100 mL) were collected immediately before feeding (0h), and 2, 4, and 6 hours after feeding. The pH analyses were performed immediately after collection, with a digital potentiometer. To determine the concentration of ammonia nitrogen (NH_3-N), 25 mL samples of rumen liquid were filtered in gauze and added to a container containing 1mL of 1:1 sulfuric acid and were stored at –10°C for further analysis. After thawing, samples were distilled with KOH_2N solution following AOAC (2015) procedures for total nitrogen.

2.5. CHEMICAL ANALYSES OF INGREDIENTS AND DIETS

At the end of the experiment, samples of ingredients, diets and leftovers were thawed and pre-dried at 55 ºC for 72 hours, and ground in a Willey-type mill (Marconi, Piracicaba, São Paulo, Brazil) with a 1.0-mm-mesh sieve and packed in closed plastic containers for chemical analyses of the DM content (method 934. 01; AOAC, 2015), ash (method 930.05; AOAC, 2015), ether extract (EE - Method 920.39; AOAC, 2015) and crude protein (CP; N × 6.25; Kjeldahl method 981.10; AOAC, 2015).

To determine neutral detergent fiber (NDF) and acid detergent fiber (ADF), the methodology described by Van Soest *et al*. (1991) was adopted, using thermostable amylase to remove starch and modified the procedure using nonwoven tissue (SENGER *et al*., 2008). The NDF content was corrected for ash and protein ($_{ap}$NDF), where the neutral detergent boiling residue was incinerated in a muffle furnace at 600°C for 4 hours. The correction for protein was performed by subtracting the neutral detergent insoluble protein (NDIP) content. The determination of lignin was performed using 72% sulfuric acid in the treatment of ADF residue. The contents of neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) were obtained following the recommendations of Licitra *et al*. (1996).

Non-fiber carbohydrates (NFC) were estimated through the equation proposed by Hall (2003): NFC (g DM/kg) = 1000 – [(CP – CPu + U) + $_{ap}$NDF + EE + ash], where CP represents the crude protein content, CPu is the crude protein derived from urea, U is the urea content, $_{ap}$NDF is the neutral detergent fiber content adjusted for ash and nitrogen compounds (MERTENS, 1997), and EE is the ether extract content.

Total digestible nutrients (TDN) of the feeds were calculated by the following equation: $TDN = CP_D + NFC_D + {}_{ap}NDF_D + 2.25 \times EE_D$, from the digestibility test, where D represents digestible nutrients.

2.6. FACIDS PROFILE OF INGREDIENTS AND DIETS

The fatty acid profile of the low-*trans* vegetable fat was analyzed before and after processing (three encapsulates) to verify the effects of temperature, alkaline pH of the urea solution and agitation on the composition.

To evaluate the fatty acids profile, fatty acid methyl esters derived from the vegetable fat and from the encapsulates were obtained using the one-step extraction method with 1.25N HCl in methanol, with 19:0 used as internal standard (SUKHIJA AND PALMQUIST, 1988). To convert the samples into fatty acid methyl esters, a basic

catalysis method followed by acid catalysis was employed as described by Oliveira *et al*. (2016). The analysis of the fatty acid methyl esters was performed by gas chromatography coupled with flame ionization detection (GC-FID, Shimadzu GC-2010 Plus, from Shimadzu Corp., Kyoto, Japan) using a 100% cyanopropyl polysiloxane capillary column (SP 2560; dimensions: 100 m of length, 0.25 mm of inner diameter, and 0.20 μm of film thickness, Supelco Inc., Bellefonte, PA). The identification of fatty acid methyl esters (FAMEs) was performed by comparing retention times to those of authentic standards (37 Component FAME Mix from Supelco Inc.) and published chromatograms (ALVES AND BESSA, 2014). In addition, the identification of fatty acid methyl esters, including branched-chain fatty acids (BCFAs), was confirmed by gas chromatography coupled to mass spectrometry (GC-MS) in a chromatograph (Shimadzu GC-MS QP 2010 Plus).

GC-FID analysis was performed with injector and detector temperatures maintained at 220 °C and 250 °C, respectively. The initial oven temperature of 50 °C was maintained for 1 minute, followed by a temperature increase of 50 °C/min up to 150 °C, which was maintained for 20 minutes. Then the temperature increased at a rate of 1 °C/min until 190 °C and finally increased at a rate of 2 °C/min until 220 °C, maintained for 30 minutes. Helium was used as carrier gas with a flow rate of 1 mL/min. For injection, 1 μL of sample (1-2 mg FAME/ml) was used in a 50:1 split ratio. GC-MS conditions, including the capillary column and GC settings, were similar to those for GC-FID analysis. The MS conditions included an ion source temperature of 200 °C, an interface temperature of 240 °C, and an emission voltage of 70 eV.

2.7. STATISTICAL ANALYSIS

The first experiment to evaluate the urea protection system was analyzed as a completely randomized design with five treatments SRU_{30}, SRU_{40}, SRU_{50}, free urea (U) and low-*tran*s vegetable fat (LTVF) with the replications being the rounds (10) of material production. Meeting the normality assumption, the data obtained were subjected to analysis of variance and Tukey's test (with 5% significance) through the PROC MIXED of SAS.

The experimental design of the 2nd experiment (metabolic cages) followed the premises of a randomized block design, in which each lamb was experimental unit (replication), and two blocks were formed based on the body weight of the animals at the beginning of the experiment. Four treatments were tested: 0.5 inclusion of free urea (U)

as control treatment and SRU_{40} encapsulated in low-*trans* vegetable fat at 1.25, 2.0, and 3.0% of total diet DM.

The data obtained were analyzed using the MIXED procedure of SAS 9.4 considering the variables block and block × treatment as random effects according to the following model:

$$Yijk = \mu + \tau i + \beta j + \tau\beta ij + \varepsilon ijk$$

Where: Y_{ijk}= value observed in the experimental unit that received treatment i, replication j; μ= general average common to all observations; τ_i = effect of treatment $_i$; βj = effect of block j; $\tau\beta_{ij}$ = effect of interaction between treatment i and block j; ε_{ijk} = random error.

The 3rd experiment (rumen parameters) followed the premises of a 4 × 4 Latin square design with experimental treatments, incubation time, animal, period, and treatment time × time, according to the following model:

$$Yijkl = \mu + Ti + Ak + Pl + (TB)ij + eijkl$$

Where Y_{ijkl} = observation of the effect of treatment i on incubation time J in period k; μ = overall mean; Ti = effect of level A (treatment, i = (free urea-U-control, SRU1.25, SRU2 and SRU3); A_k = effect of animal (k = 1, ..., 6), P_l = effect of period (collection time 0, 2, 4 and 6 h post-morning feeding); AB_{ij} = effect of interaction between treatment i (level) and time j; e_{ijkl} = random error associated to each observation.

The collected data underwent analysis of variance using the PROC MIXED command in SAS. Mean comparisons were conducted through orthogonal contrasts, which were established using the PROC IML. These designated contrasts aimed to independently evaluate the control treatment (free urea U0.5) versus SRU1.25. This comparison was made because they had the same theoretical urea quantity but differed in terms of being free urea versus protected urea. The purpose was to investigate the impact of vegetable fat on urea protection efficiency. Additionally, linear and quadratic contrasts were examined across the three SRU inclusion levels (1.25%, 2%, and 3%). Statistical significance was determined at a threshold of $P < 0.05$.

3. RESULTS

All formulations showed encapsulation yields above 80%. SRU_{40} showed the highest encapsulation efficiency (85.5%), retaining the highest percentage of U when compared to SRU_{30} and SRU_{50}, showing that the proportion of 40% urea and 60% LTVF

was the most efficient even though it had higher moisture and intermediate CP concentration (115%) compared to the other formulations (Table 3).

Table 3. Yield and efficiency of microencapsulation formulations, crude protein, and moisture of microparticles of slow-release urea (SRU) produced from lipid matrix of low-trans vegetal fat (LTVF) at different proportions.

Item (%)	Slow-Release Urea (SRU) [1]			SEM[2]	*P*-value[3]
	SRU_{30}	SRU_{40}	SRU_{50}		
Microencapsulation yield (%)	82.9 c	85.5 a	83.7 b	0.02	0.023
Microencapsulation efficiency (%)	96.0 b	98.0 a	96.6 b	0.49	0.002
Crude protein (%)	80.2 c	115 b	134 a	2.74	0.001
Moisture (%)	5.68 b	6.21 a	5.31 b	0.72	0.039

[1]SRU_{40} = slow-release urea produced from 70, 60 and 50% of lipid matrix of low-*trans* vegetal fat (LTVF) and 30, 40 and 50% of urea (U), respectively (SRU_{30}; SRU_{40}; SRU_{50}).
[2]SEM=standarderrormean
[3]Means followed by the same letters do not differ according to Tukey's test; significant at $P \leq 0.05$.

In the TG (Figure 1a) and DSC (Figure 1b) thermal analyses of the encapsulating material (LTVF) and core material (U) used in the processing, it was observed that U showed two main thermal degradation events, with T_{onset} of 187 °C in 1017 s (16.95 min), while LTVF showed a single degradation event with T_{onset} of 381 °C in 2164 s (36.06 min). When examining the slow-release urea formulations (SRU_{30}, SRU_{40}, and SRU_{50}), it was observed that all SRU showed two main thermal degradation events. T_{onset} temperatures for these formulations were 165 °C in 851 seconds (14.18 min) for SRU_{30}, 168 °C in 866 seconds (14.43 min) for SRU_{40}, and 160 °C in 832 seconds (13.86 min) for SRU_{50}.

SRU_{40} formulation was the only one that exhibited an event prior to thermal degradation, which can be attributed to a higher moisture content (6.21%) when compared to the other SRUs. LTVF proved to be an efficient encapsulating agent for urea, with SRU_{40} showing the highest initial temperatures and degradation times. On the other hand, SRU_{50} formulation, with a higher urea inclusion level, showed lower initial temperatures and degradation times. This effect is reaffirmed by T_{max} of 202 °C in 1081 s (18.01 min), 208 °C in 1125 s (18.75 min) and 192 °C in 1035 s (17.25 min) for SRU_{30}, SRU_{40} and

SRU_{50}, respectively, which means that higher temperature and time are required for the highest degradation rate to occur for SRU_{40} and lower for SRU_{50}.

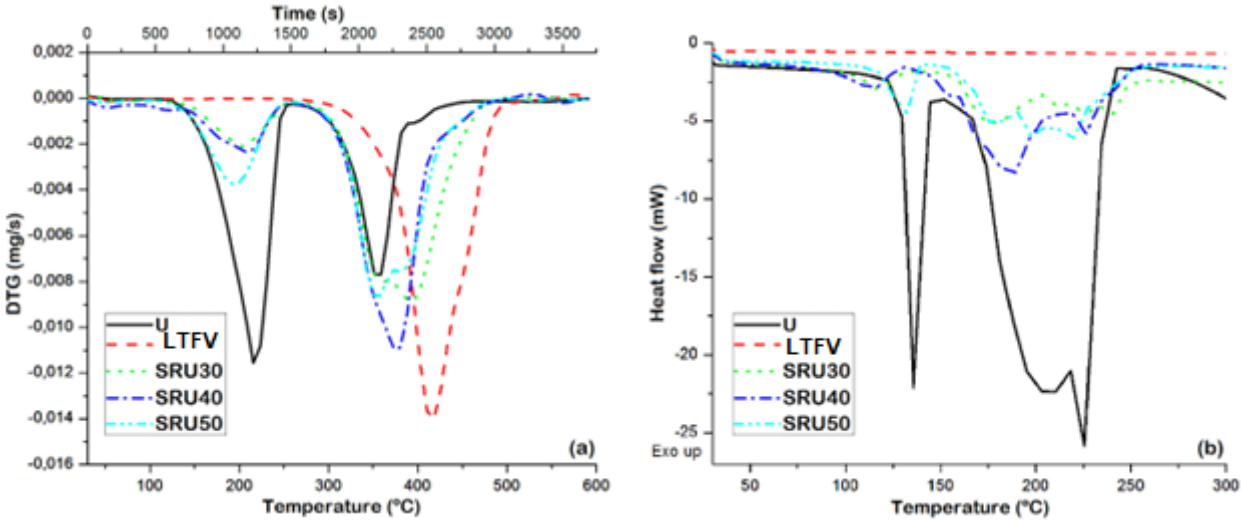

Figure 1. (a) Thermogravimetric curves (DTG curves) and (b) Differential scanning calorimetry curves (DSC curves) for free urea (U), low-*trans* vegetable fat (LTVF) and urea systems protected by vegetable fat (SRU_{30}, SRU_{40}, and SRU_{50}).

In terms of thermal behavior in DSC, urea showed one endothermic event corresponding to its melting point at 135 °C, as well as two overlapping endothermic events with peaks at 206 °C and 226 °C, associated with its thermal degradation. In contrast, LTVF showed no thermal events within the evaluated temperature range of 30 to 300 °C. For the protected urea formulations, the melting event of LTVF was not observed, indicating that it was already in a molten state, as seen in the analysis of individual components, whereas the events including the initiation of thermal degradation of urea in the protected urea formulations showed peak temperatures at 181, 211, and 225 °C for SRU_{40}, 189 and 226 °C for SRU_{40}, and 175, 198, and 220 °C for SRU_{50}.

The manufacturing process of the slow-release urea changed the fatty acid profile (Table 4) of the LTVF used as a wall to protect urea, which caused reduction mainly in C18:2n-6 and C18:3n-3 and increase in C18:1-t, C18:1-cis and C18:2. However, the different concentrations of LTVF in the three formulations did not change the fatty acid profile.

Due to the results of the first phase of the experiment, mainly based on the thermal analyses, which showed a more gradual degradation of the core, protected SRU_{40} was chosen to perform the *in vivo* tests on sheep feeding.

Table 4. Fatty acids profile (g/100 g total FA) of low-trans vegetal fat (LTVF) and different formulations of slow-release urea (SRU) produced from the lipid matrix of LTVF.

FA composition	Vegetable fat	Slow-release urea (SRU)[1]		
		SRU_{30}	SRU_{40}	SRU_{50}
C14:0	0.15	0.08	0.08	0.08
C16:0	14.4	12.6	12.0	11.6
C16:1- *cis* 9	0.07	0.08	0.10	0.06
C17:0	0.07	-	0.04	0.02
C18:0	4.15	7.05	5.93	4.97
C18:1- *trans* others	-	12.4	11.9	11.3
C18:1- *cis* 9	25.9	28.3	29.1	30.1
C18:1- *cis* 11	1.15	1.91	1.99	1.94
C18:1-*cis* others	-	5.65	5.89	5.78
C18:2 others	-	3.94	4.18	4.39
C18:2n-6	48.2	26.0	26.8	27.7
C20:0	0.34	0.35	0.34	0.31
C18:3n-3	5.06	1.20	1.26	1.24
C22:0	0.37	0.36	0.37	0.35
C24:0	0.11	0.13	0.12	0.13

[1]SRU_{40} = slow-release urea produced from 70, 60 and 50% of lipid matrix of low-*trans* vegetal fat (LTVF) and 30, 40 and 50% of urea (U), respectively (SRU_{30}; SRU_{40}; SRU_{50}).

The different forms of urea offered (free and protected) and the inclusion levels of SRU40 did not alter DM intake (P = 0.924), which was on average 846 g/day. N-ingestion (Figure 2.b) was also not affected by how urea was supplied (P = 0.890) nor by the inclusion level of SRU_{40} in the diet (P = 0.481), averaging 19.7 g/day.

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Regarding the nitrogen (N) metabolism, losses through fecal (P = 0.014) and urinary (P = 0.004) routes changed from the urea encapsulation for slow release, showing

higher N-excreted via urine and fecal route in lambs fed free urea ($U_{0.5}$), which resulted in lower N-retention (P = 0.028). As for SRU inclusion levels, there was no difference in N-fecal excretion (P = 0.944) in lambs, however there was a linear reduction in N-urinary excretion (P = 0.032) for lambs fed higher levels of SRU, impacting a linear increase in N-retention (P = 0.019) with maximum value when 3% SRU (as DM) was provided (9.29 g/day of N).

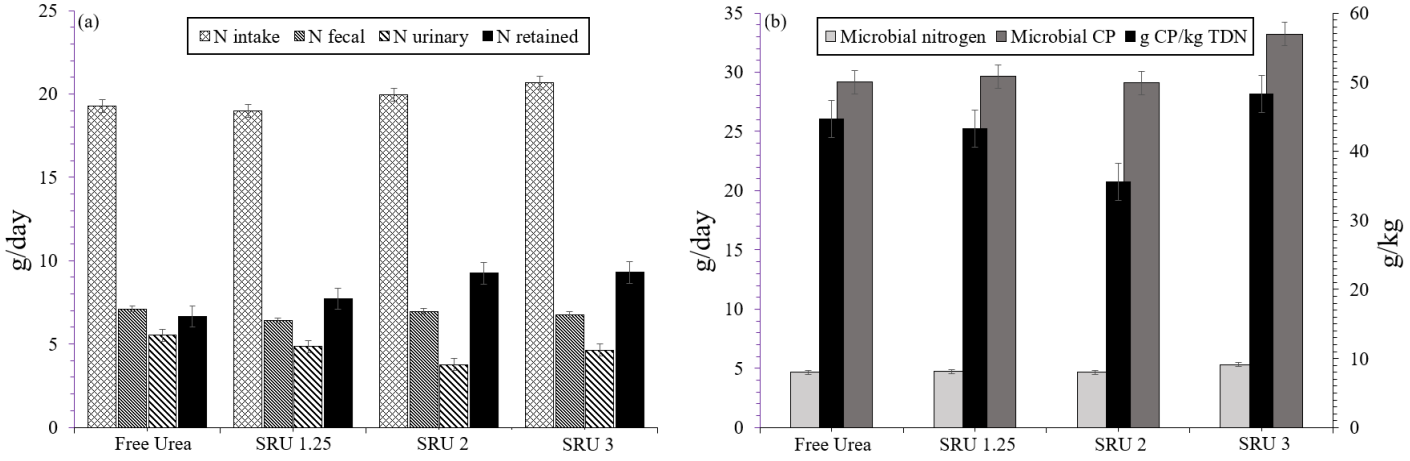

Figure 2. (a) Means of nitrogen (N) metabolism variables, N-intake, N-fecal and N-urinary excretion and N-retained (g/day); and (b) Means of microbial nitrogen and microbial protein production (g/day) and N-microbial production/TDN (energy) intake ratio in lambs (n = 32) fed slow-release urea (SRU40) core protected by the low-trans vegetable fat matrix (40%U: 60%LTVF ratio) and added in 1.25% (SRU1.25), 2% (SRU2) and 3% (SRU3) in comparison to free urea (U).

N-microbial (P = 0.014) and CP-microbial (P = 0.014) productions linearly increased due to the inclusion of SRU in the lambs' diet, but there was no effect of the urea encapsulation for slow release (P = 0.582) on microbial production efficiency (g MicProt/TDN Intake).

The inclusion of SRU linearly increased the concentration of BUN (P = 0.009) and tended to linearly increase the concentration of total proteins (P = 0.096) and AST (P = 0.092). $SRU_{1.25}$ promoted lower BUN concentration (P = 0.045) compared to control ($U_{0.5}$), and $SRU_{1.25}$ tended (P = 0.059) to have lower serum magnesium concentration compared to $U_{0.5}$ (Table 5). Serum concentrations of albumin, GGT, creatinine, cholesterol, triglycerides, calcium, and phosphorus were not affected by urea encapsulation for slow release.

Blood urea nitrogen concentrations (BUN, Figure 3.a) in lambs showed a linear decrease in relation to the form of offer, with the highest BUN concentrations for $U_{0.5}$

(control) at 0, 4 and 6 h after feeding (P = 0.010; P = 0.001; P = 0.001 respectively). All diets with SRU caused peak after 2 h of feeding, showing the same concentration as the diet with $U_{0.5}$ (P = 0.117). However, it is possible to observe from the graphical representation that the diets with SRU showed the lowest values and variations of BUN concentration over time in comparison to the diet with free U.

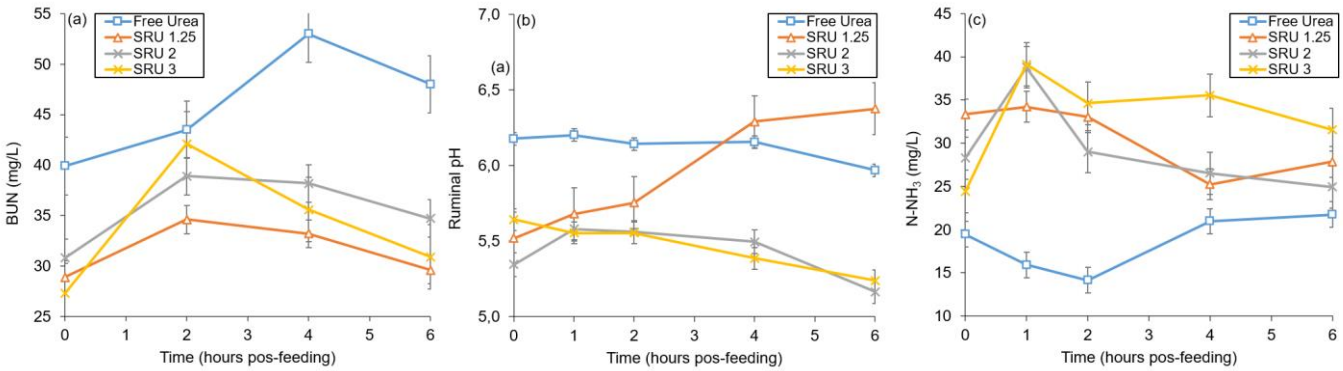

Figure 3. Means over hours post-feeding of blood urea nitrogen (BUN) (a) rumen pH (b) and ammonia nitrogen (N-NH3) (c) in lambs (n = 32) fed slow-release urea (SRU40) core protected by the low-trans vegetable fat matrix (40% of U: 60% of LTVF ratio) and added in 1.25% (SRU1.25), 2%(SRU2) and 3%(SRU3) in comparison to free urea (U).

Regarding rumen parameters, the inclusion of SRU altered the rumen pH (Figure 3.b) and ammonia nitrogen concentration (Figure 3.c), especially in the post-feeding period. Rumen pH decreased linearly 1 h after feeding (P = 0.044) as well as 4 h (P = 0.008) and 6 h (P = 0.006), however it did not vary at 2 h (P = 0.246). There was no difference in NH_3-N concentration before feeding (P = 0.368), but there was a linear increase at 1, 2, 4 and 6 h after the first feeding (P = 0.010; P = 0.002; P = 0.019; P = 0.022 respectively)

4. DISCUSSION

The high encapsulation yields (above 82%) and efficiency (95%) shown by all systems, especially the SRU_{40} formulation, demonstrate the suitability of the melt emulsification technique in encapsulating urea using LTVF as protection material. These results indicate that fusion emulsification can efficiently protect urea during processing, avoiding significant losses. Furthermore, LTVF proved to be stable as protection material, showing no endothermic events when evaluated alone, which confirms its suitability for encapsulation.

However, when LTVF was compared with other lipid materials used as encapsulating agents, such as beeswax and carnauba waxes, the waxes showed higher yields (DE MEDEIROS *et al*., 2019; CARVALHO *et al*., 2019). Carvalho *et al*. (2019) tested a 50:50 ratio of urea: beeswax and achieved an efficiency of 92.5%. However, Netto *et al*. (2021) tested urea protected by carnauba wax in the proportions of 50:50 and 25:75 and found that when reducing the proportion of urea incorporated, the yield also varies, going from 92.1% to 87.5% and linked this reduction to the higher viscosity of wax, which caused loss of material by adherence on the walls of the containers during the preparation process, which does not happen with LTVF for bring less viscous.

During the production of SRU, it was necessary to add water to solubilize the urea and allow emulsification with LTVF, using lecithin as an emulsifier. However, during the dehydration process in the oven, water is removed from the systems. This removal of water can lead to nitrogen losses, probably due to evaporation of the water carrying some of the nitrogen present in the urea. These losses explain why encapsulation efficiencies are less than 100% and why there is still residual moisture in the final material. Although complete water removal was not achieved in any of the formulations, due to the hygroscopicity of the urea and the barrier formed by the LTVF that prevents the total evaporation of the water, the moisture content present in the final material was not harmful, as reaffirmed by the thermal analyses.

When the thermal degradation events of the formulations were analyzed, it was observed that they are a combination of the degradation patterns of urea and LTVF. This combination of events indicates that all formulations used to encapsulate urea with vegetable fat were efficient, as there was an increase in the temperature of the main degradation stage associated with urea degradation. However, the SRU_{40} formulation was more efficient in increasing this temperature when compared to SRU_{30} and SRU_{50}.

Regarding the DSC results, it can be suggested that SRU_{40} has better thermal stability, explained by the fact that the events started to happen at a higher temperature and only at two moments, unlike SRU_{30} and SRU_{50} that presented three events initiated at lower temperatures. Moreover, the higher stability corroborates with what was observed in the TG and DTG curves. This better thermal stability for SRU_{40} may indicate a slower release of urea into the rumen environment and be associated with the limit on the amount of core that LTVF can incorporate, as there was a negative effect with increasing urea incorporated in the SRU_{50} formulation. This worsening in the stability of SRU_{50} with 50% U and 50% LTVF, may also have occurred due to two main factors: the

higher water content that was added into the system in the form of urea solution, and the lower emulsifier content, as this was added into the formulation at 1% relative to the mass of LTVF.

The fact that the LTVF melts at the typical room temperature at which the experiment was performed, indicates that there may be a lower strength of the emulsion. According to Jiang *et al*. (2018) an interfacial membrane of the emulsion with lower strength can easily be subject to the perforation of fat crystals, and the distortion and rupture of some emulsion droplets, which consequently can cause phase separation between the encapsulating material and the core material, compromising the protection of the SRU.

Another factor that may interfere with this stability is that once manufactured, emulsions evolve to full separation naturally by the effect of coalescence and Ostwald ripening, on time scales that can vary from hours to years (BIBETTE *et al*., 2002). Thus, studying the materials during storage allows one to plan any processing adjustments and to define the best form of storage that is needed to ensure the stability of the emulsion obtained and the maintenance of urea protection. However, this issue of phase separation, should it occur during storage, was not noticed during the experiment due to the almost immediate use of the materials after manufacturing, without long waiting periods that would allow better observation.

When comparing the fatty acid composition of the isolated and encapsulated LTVF, a reduction in polyunsaturated fatty acids from the vegetable fat was observed post encapsulation processing. This may be related to the interaction between Urea and LTVF during the formation of the encapsulation matrix because of the heating of the vegetable fat in an alkaline medium, since the urea solution is at 60°C had a pH of 9.2. LTVF comes from the hydrogenation of soybean oil, and this effect can be explained by the fact that soybean lipids are easily *trans*-esterified during stirring at 60°C in alkaline medium, leading to the breakdown of unsaturated bonds (HAAS *et al*., 2004).

The increase in *trans*-fat caused by processing the material, and the concerns that this could increase the *trans* fatty acid of the milk or meat, may be a contra-indication. However, it is important to consider that this lipid profile can still suffer modifications in the rumen of animals due to the biohydrogenation processes promoted by rumen microorganisms (LARQUÉ *et al*., 2003). Therefore, it becomes necessary to further study if the fatty acid composition of these slow-release urea products can affect the milk and meat quality of animals fed with this material. Thus, the information on changes in the

fatty acid profile produced by this study is insufficient for not recommending the use of LTVF for urea protection, since it was efficient in protecting urea.

The diets used in the *in vivo* trial with fistulated animals were formulated to be iso-nitrogen, aiming to meet the same weight gain requirement. Consequently, it was expected that there would be no significant differences in dry matter intake and nitrogen content ingested, which in fact was observed in the experiment. These results indicate that the addition of SRU_{40} did not affect the animals' intake, either because of the increased ether extract due to LTVF, or because of the higher urea content added to the diet and the consequent reduction in soybean meal.

Ruminant diets containing more than 1% urea may present low palatability and hence reduced intake (WILSON *et al*., 1975). Moreover, urea-rich diets may lead to excessive ammonia nitrogen in the rumen and accumulation of urea nitrogen in the blood, leading to toxicity in ruminants (KERTZ *et al*., 1982). However, these factors did not interfere with the animals' consumption during the present experiment, which is explained by the lower amount of serum urea in the animals fed protected urea. However, the NH_3-N was higher for the diets with SRU_{40}, but this effect may not have occurred due to the greater constancy over time of this concentration promoted by the slow release of urea.

Because of the greater constancy of ammonia nitrogen production in the rumen promoted by SRU_{40}, there was also greater stability in the amount of blood serum urea. Previous studies, such as Kaneko *et al*. (2008), established the physiological values of blood serum urea for sheep between 17 and 43 mg/dL. In the SRU_{40} treatments, BUN values always remained within this physiological range, even for the diet with the highest level of protected urea, which corresponded to 3% SRU_{40}, equivalent to 1.2% free urea as DM. In contrast, the diet with free urea showed values that exceeded the physiological limit at 2, 4 and 6 h, but no animal showed clinical signs of intoxication during the experimental period.

Results showed that the use of LTVF as a urea protection agent, providing its slow release, was efficient in reducing the urea hydrolysis peak, reaffirming the results obtained in the thermal analyses that showed that SRU_{40} was the most stable formulation in the DSC analysis. This resulted in a more even distribution of urea release over time, which positively impacted ammonia utilization, as demonstrated by Geron et al. (2016) and de Medeiros *et al*. (2019).

This constancy in both NH_3-N in the rumen and BUN can also be attributed to higher efficiency of urea recycling, which in growing ruminants is regulated by N intake. To facilitate this nitrogen transfer, mechanisms present in the kidney and GI tract can recover excreted urea and redirect it to the GI tract, where rumen bacteria take better advantage of the nitrogen coming from blood urea (MARINI AND VAN AMBURGH, 2003).

Possibly, this strategy allowed the rumen microorganisms to optimize the utilization of ammonia for bacterial protein synthesis, as microbial protein production by the microorganisms increased, especially in comparison to the increased N-retention and the consequent reduced excretion through feces and urine. N-retention is an essential indicator of the body's ability to utilize and synthesize protein from available nitrogen. The data obtained showed that animals fed the diet containing SRU_{40} showed higher nitrogen retention when compared to those fed the $U_{0.5}$.

This increased efficiency in N metabolism brings benefits not only to animal performance, but also to the environment. The reduction in N excretion via feces and urine is important because excess N-excreted can pose an environmental risk, especially when it accumulates in the soil and can lead to contamination of water resources (TAMMINGA, 1996; RIDOUTT *et al*., 2017). Therefore, the use of LTVF-protected urea, such as SRU, has the advantage of also mitigating environmental impacts associated with ruminant production.

In addition, by replacing soybean meal with the protected urea without harming the metabolism of animals, there is the possibility for the producer to have financial advantages by reducing feed costs without compromising animal performance and reducing the impacts of seasonal soybean market fluctuation on their production.

5. CONCLUSION

Low-*trans* vegetable fat was efficient in encapsulating urea, especially the formulation in the proportion of 40% urea and 60% vegetable fat (SRU_{40}) for presenting higher yield and efficiency of encapsulation and greater thermal stability.

SRU_{40} formulation can also be used in sheep's diets with an inclusion of up to 3% in dry matter, being efficient in replacing soybean meal and reducing nitrogen excretion into the environment without affecting the intake of DM, improving the use of dietary nitrogen without causing toxicity, being a nutritional strategy for the protection of urea

that promotes productive efficiency and market competitiveness, besides contributing to environmental preservation and sustainability of livestock production.

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CHAPTER 2

Effect of the inclusion of slow-release urea in the rumen by microspheres of vegetable fat on carcass quality and the physicochemical composition and fatty acid profile of lamb meat.

EFFECT OF THE INCLUSION OF SLOW-RELEASE UREA IN THE RUMEN BY MICROSPHERES OF VEGETABLE FAT ON CARCASS QUALITY AND THE PHYSICOCHEMICAL COMPOSITION AND FATTY ACID PROFILE OF LAMB MEAT

ABSTRACT

This study evaluated the effect of including slow-release urea protected with hydrogenated vegetable fat (SRU) in the diet of lambs on carcass characteristics and physicochemical composition of the meat. Thirty-two non-castrated Santa Inês lambs, with an average age of four months and initial body weight of 17.98 ± 2.01 kg, were used in a randomized block design, with the initial weight used as the criterion for forming two blocks, four treatments, and six replicates. The tested diets were divided into the control treatment, with 0.5% free urea (U0.5) in dry matter, and the other treatments consisted of SRU40 included at proportions of 1.25% (SRU1.25), 2.0% (SRU2), and 3.0% (SRU3) of the total diet dry matter. The diets were formulated to meet the requirements of growing male sheep for an average daily gain of 200 g/day, respecting a forage:concentrate ratio of 30:70. The forage source used was Tifton-85 hay (Cynodon sp), and the concentrate was composed of ground corn, corn silage moistened with palm mucilage, soybean meal, mineral salt, and free urea or SRU40. The experiment lasted a total of 75 days, with 15 days of adaptation and 60 days of performance evaluation. The animals were weighed at the beginning and end of the experiment after a 16-hour fast. After slaughter, the animals were stunned, bled, skinned, and eviscerated. The pH and weight of the carcasses were measured before and after cooling, and carcass yields were calculated. The Longissimus lumborum muscles were collected and analyzed for physicochemical composition and fatty acid profile. The inclusion of SRU40 in the lamb's diet did not affect the intake of DM ($P = 0.924$) and CP ($P = 0.948$), but there was a linear increase in the intake of EE ($P = 0.001$) and GE ($P = 0.023$). Weight gain and carcass characteristics were not altered ($P > 0.05$). The centesimal composition of the meat also remained constant ($P > 0.05$). There was a linear increase in the concentration of CLA and the isomers of 18:1 (cis-12, cis-13, and cis-15) and branched-chain fatty acids, resulting in a linear increase in BCFA content ($P = 0.048$). The total trans-MUFA increased linearly ($P = 0.003$), and the total PUFA also increased linearly, reflecting the increase in n-3 PUFA ($P = 0.037$). Hydrogenated vegetable fat was effective in protecting urea, allowing the substitution of soybean meal in the diet of sheep without compromising meat quality or carcass weight. Despite

containing trans-fatty acids, ruminal biohydrogenation and the increase of polyunsaturated fatty acids in the meat compensated for the negative effects, making this approach promising for improving competitiveness and consumer satisfaction in meat production.

KEYWORDS: Fatty acids, nitrogen, trans fat.

1. INTRODUCTION

Partial protection of urea is a strategy used to maximize its efficiency in ruminant feeding. Several methods, such as encapsulation, microencapsulation, and emulsion, can be employed using a variety of polymers or lipid sources. These approaches aim to gradually release urea in the rumen, minimizing the risk of toxicity and improving nutrient degradation synchronization. Recently, some studies have explored promising alternatives, such as using polymeric microparticles of calcium pectinate, demonstrating significant improvements in nitrogen utilization efficiency and reducing the risk of poisoning in sheep (MELO *et al*., 2021).

Other researchers have chosen to use materials such as waxes, including beeswax and carnauba wax, for the microencapsulation of urea due to their stability in the ruminal environment (CARVALHO *et al*., 2019; MEDEIROS *et al*., 2019). However, these materials may pose challenges related to availability and processing complexity, hindering large-scale applications and increasing costs. Therefore, it is necessary to continue seeking more accessible alternatives for the partial and effective protection of urea in the context of ruminant feeding.

Vegetable fat (VF) emerges as an alternative to the waxes already used for urea protection, as it is readily available and easy to process, with desirable physicochemical characteristics for use as an encapsulant. One of these characteristics is a higher concentration of saturated fatty acids and a lower concentration of polyunsaturated fatty acids due to the hydrogenation process, as well as the ability to trap air during the product formation process, physically interfering with protein particle continuity and easily emulsifying the liquid (PIZARRO *et al*., 2013). This may indicate that its composition can reduce VF toxicity to ruminal microorganisms and render it inert in the ruminal environment.

However, VF contains trans fatty acids in its composition, which have adverse effects on human health. The consumption of these fatty acids in humans can result in increased plasma lipid levels, promoting inflammation and arterial cell calcification, representing a known risk factor for coronary heart disease (KUMMEROW, 2009; SALTER, 2013). It is worth noting, however, that the fatty acids consumed by ruminants undergo biohydrogenation in the rumen, which can alter the fatty acid profile deposited in the meat (HARFOOT; HAZLEWOOD, 1997), and thus has the potential to reduce the

concentration of trans fatty acids derived from VF that would be deposited in the meat and consumed by humans.

In addition to the fatty acid composition of ingredients in the diet, it is important to consider the effect of using high proportions of concentrates in finishing formulations. The high presence of concentrates can lead to a reduction in ruminal pH and an increase in the occurrence of intermediate fatty acids from biohydrogenation, such as conjugated linoleic acid (CLA) isomers, *trans*-10, *trans*-9, and *cis*-12 (PERFIELD *et al*., 2007). However, according to Loor *et al*. (2004), these changes in biohydrogenation are independent of pH changes but still relate to high levels of grain or concentrate reaching the rumen, which may induce a shift in *trans*11-18:1, the main *trans*-18:1 intermediate, to *trans*10-18:1. Therefore, it is essential to understand changes in the meat fatty acid profile resulting from the inclusion of slow-release urea protected with VF in diets with a high proportion of concentrate.

Considering the advantages of hydrogenated vegetable fat as a potential wall material for urea protection in the ruminal environment, along with its favorable characteristics of easy availability, processing, physicochemical stability, and fatty acid composition, we hypothesized that the inclusion of slow-release urea protected with VF in sheep feeding, replacing soybean meal, will result in a gradual release of urea in the ruminal environment, promoting better synchronization and degradation of nutrients in the rumen without affecting the biohydrogenation process and without compromising carcass characteristics, meat quality, and meat fatty acid profile in lambs.

Therefore, the objective of this study was to evaluate the effects of the inclusion of slow-release urea protected with VF compared to conventional urea in lambs feeding on carcass characteristics, meat quality, and meat fatty acid profile.

2. MATERIAL AND METHODS

2.1. OBTENTION OF SLOW-RELEASE UREA (SRU_{40})

For the production of rumen slow-release urea, vegetable fat was used as the encapsulating material. It comprised vegetable oils (mainly soybean oil), antioxidants (TBHQ and citric acid), and the antifoaming agent dimethylpolysiloxane. It was characterized by a minimum smoke point of 225°C (Cukin vegetable fat, Bunge Alimentos S.A.). The ratio used for the core (urea) and wall material (VF) was 40:60

(SRU_{40}). The encapsulation process was carried out using the Fusion-Emulsification technique, as described by Medeiros *et al*. (2019), with soy lecithin employed as the emulsifying agent at a ratio of 1% relative to the mass of VF.

VF was weighed on an analytical balance and added to a beaker to produce the material. Subsequently, a surfactant (40% soy lecithin) was added to the beaker at a ratio of 1% of the mass of the vegetable fat. VF and lecithin were maintained in a thermostatic bath at a temperature of 60°C. In another beaker, urea was dissolved in distilled water to form a 50% (w/w) solution with a pH of 9.2. The solution was also kept in the thermostatic bath to facilitate urea dissolution and to equalize the temperature with HVF.

After the temperature of the materials stabilized, the urea solution was gradually added to the beaker containing HVF and soy lecithin while mixing with a dispersers homogenizer crushing (T25 digital Ultra-Turrax®, Ika, USA). Finally, the emulsion was transferred to plastic containers and kept in a forced air circulation oven at a constant temperature of 55°C for 24 hours for dehydration. After the material was dried, it was removed from the oven and, upon reaching room temperature, stored in a refrigerator at 2°C for subsequent analysis and use.

2.2. ANIMAL MANAGEMENT, DIETS, EXPERIMENTAL DESIGN

For the animal trial, all animal experiments, all management practices were performed only after approval (Protocol number 58/2021) and in strict concordance with the recommendations of the Ethics Committee on Animal Use (CEUA) of the Federal University of Campina Grande, Paraíba, Brazil.

Thirty-two non-castrated Santa Inês lambs, with an average age of four months and an average body weight of 17.98 ± 2.01 kg, were used in a randomized complete block design. They were grouped into two blocks based on their initial weight, with four treatments and six replications. The control treatment had 0.5% free urea (UL0.5) in DM, and the other treatments consisted of SRU_{40} added to the animal's diets at proportions of 1.25% (ULL1.25), 2.0% (ULL2), and 3.0% (ULL3) of the total diet DM, which corresponded, respectively, to 0.5 (similar to the control treatment), 0.8, and 1.2% free urea in the diet.

The lambs were weighed before the beginning of the experiment while fasting, identified, vaccinated against clostridiosis (Biovet Resguard Multi®, São Paulo, Brazil), orally dewormed with a 5% Levamisol hydrochloride-based dewormer (Ripercol® L, São

Paulo, Brazil), and supplemented with a vitamin mix (A, D, and E). The animals were individually housed in pens equipped with a drinking trough and a feeder. The experiment lasted for 75 days, with 15 days of adaptation and 60 days of performance evaluation.

The diets were formulated to meet the requirements of growing male lambs for an average daily gain of 200 g/day, according to NRC (2007) guidelines. The diets were formulated with a forage-to-concentrate ratio of 30:70. The forage source used was Tifton-85 hay (Cynodon sp.), and the concentrate was composed of ground corn, corn grain silage hydrated by cactus mucilage (HCS), soybean meal, mineral salt, and free urea or SRU_{40} (Table 1).

Table 1. Chemical and fat acid composition of the ingredients used in the experimental diets.

Item (g/kg DM)	Ground corn silage[1]	Free urea	SRU_{40}[2]	Soybean meal	Ground corn	Tifton–85 hay
Dry matter (g/kg as fed)	670	980	981	916	899	872
Crude ash	18.1	2.10	0.84	80.7	14.5	81.2
Crude protein	97.1	2784	1147	402	87.9	88.3
Ether extract	39.4	-	595	15.5	72.9	11.0
$_{ap}$Neutral detergent fiber[3]	128	-	-	157	115	729
Non-fiber carbohydrates	718	-	-	345	710	91.3
Cellulose	105	-	-	89.2	75.7	321
Hemicellulose	19.4	-	-	59.6	31.0	355
Acid detergent lignin	3.22	-	-	8.23	8.32	52.5
Fatty acid composition (g/100g FAME)[4]						
C14:0	0.00	-	0.08	0	0	1.09
C16:0	18.4	-	12.0	18.1	16.0	32.9
C16:1c9	0	-	0.10	0	0	0
C17:0	0.53	-	0.04	0	0	0
C18:0	5.62	-	5.93	7.38	5.84	9.48
C18:1-trans outros	0	-	11.9	0	0	0
C18:1-c9	34.1	-	29.1	16.1	33.1	9.22
C18:1-c11	1.05	-	1.99	1.66	0.66	0.90
C18:1-cis outros	0	-	5.89	0	0	0
C18:2 outros	0	-	4.18	0	0	0
C18:2 n-6	38.6	-	26.8	50.8	42.1	12.7
C20:0	0.95	-	0.34	0.37	0.73	2.01
C18:3 n-3	0.81	-	1.26	4.90	1.48	27.5

C22:0	0	-	0.37	0.58	0	2.01
C24:0	0	-	0.12	0	0	2.25

[1]Hydrated with cactus pear mucilage.
[2]SRU_{40} = slow-release urea produced from 60% lipid matrix of low-*trans* vegetal fat (LTVF) and 40% urea (U);
[3]$_{ap}$NDF, Neutral detergent fiber tested with heat stable amylase and corrected for ash and nitrogen compounds;
[4] Fatty acid methyl ester.

To serve as a soluble carbohydrate source and enhance the use of urea in the rumen, corn grain silage moistened with prickly pear forage mucilage (*Opuntia stricta* Haw.) was added and mixed at a ratio of corn grain to prickly pear mucilage of 75:25%. Free urea and SRU_{40} were added to the concentrate and mixed in a Y-mixer (Table 2).

Table 2. Ingredient proportion, chemical and fatty acid composition of experimental lamb diets including slow-release urea (SRU) produced from the lipid matrix of vegetal fat.

Item	Free U (%DM)	SRU_{40}[1] (%DM)		
	0.5	1.25	2.0	3.0
Ingredients				
Tifton–85 hay	300	300	300	300
Ground corn	505	498	518	539
Ground corn silage[2]	20	20	20	20
Soybean meal	140	139.5	112	81
Free urea (U)	5.0	-	-	-
Slow-release urea (SRU40)[2]	-	12.5	20	30
Mineral mixture[3]	30	30	30	30
Chemical composition of diet (g/kg)				
Dry matter (g/kg as fed)	892	893	893	893
Crude ash	73.3	73.2	71.2	69.1
Crude protein	142	142	141	142
Ether extract	43.1	49.7	55.5	62.5
$_{ap}$Neutral detergent fiber[4]	301	300	298	295
Non-fiber carbohydrates	448	443	448	452
Cellulose	131	131	130	128
Hemicellulose	149	149	148	146
Acid detergent lignin	21.2	21.1	21.1	21.0
Neutral detergent insoluble protein[4]	312	310	312	314
Acid detergent insoluble protein[4]	194	192	196	199
Total digestible nutrients[5]	806	814	829	846
Metabolizable energy, MJ/kg[8]	2.98	3.01	3.06	3.12
Fatty acid composition (g/100g FAME)				

C14:0	0.084	0.085	0.083	0.082
C16:0	17.461	16.696	16.266	15.842
C17:0	0.010	0.013	0.015	0.017
C18:0	6.196	6.162	6.124	6.090
C18:1-trans outros	0	1.707	2.549	3.395
C18:1-c9	30.461	30.236	30.326	30.386
C18:1-c11	0.735	0.916	0.996	1.077
C18:1-cis outros	0	0.847	1.264	1.683
C18:2 outros	0	0.600	0.896	1.193
C18:2 n-6	40.249	38.297	37.313	36.324
C20:0	0.814	0.747	0.714	0.681
C18:3 n-3	3.634	3.316	3.078	2.852
C22:0	0.184	0.212	0.216	0.223
C24:0	0.172	0.167	0.160	0.153

[1]SRU_{40} = slow-release urea produced from 60% lipid matrix of low-trans vegetal fat (LTVF) and 40% urea (U).
[2]Ground corn silage hydrated with cactus pear mucilage;
[3]Assurance levels (per kilogram of active elements): 120 g of calcium, 87 g of phosphorus, 147 g of sodium, 18 g of sulfur, 590 mg of copper, 40 mg of cobalt, 20 mg of chromium; 1,800 mg of iron, 80 mg of iodine; 1,300 mg of manganese, 15 mg of selenium; 3,800 mg of zinc, 300 mg of molybdenum; maximum 870 mg of fluoride
[4]apNDF, Neutral detergent fiber tested with heat stable amylase and corrected for ash and nitrogen compounds (g/kg CP);
[7]Total digestive nutrients determined *in vivo* digestibility experiment;
[8] Calculated according to Weiss (2020).

A prior adaptation to urea was performed, gradually introducing it during the adaptation period. The diet was offered as a total mixed ration (TMR) in two equal portions to the lambs at 7:00 a.m. and 5:00 p.m. and was adjusted daily based on the weighing of leftovers from the previous day to ensure a leftover between 10% and 20%. Water was provided ad libitum.

Samples of leftovers were collected every 3 days to create composite samples that were analyzed to determine nutrient intake. To evaluate microbial protein production, spot urine samples were collected in the middle of the experimental period. Purine derivatives (PD) excreted were determined by summing the daily excretion of allantoin and uric acid in urine, excluding xanthine and hypoxanthine excretion. This approach is adopted due to the high correlation between the sum of allantoin and uric acid with nucleic acid concentration in the rumen (TOPPS; ELLIOTT, 1965), with xanthine and hypoxanthine values in small ruminants generally representing less than 1% of the total PD. The absorption of PD was calculated based on the mathematical model described by Chen *et al*. (1990). Microbial nitrogen supply (MNS) was estimated according to Chen *et al*.

(1992), and microbial protein synthesis (MPS) was calculated by multiplying MNS by 6.25.

For performance trail, the animals were weighed at the beginning and end of the experiment after a 16-hour fast. Subsequently, after the final weighing, the animals were slaughtered. During slaughter, the animals were stunned using a pneumatic gun, then bled out through the cutting of the jugular veins and carotid arteries, and subsequently skinned and eviscerated. The carcasses were hung using hooks through the Achilles tendon, maintaining the metatarsal joints spaced at 17 cm. Before the carcass entered the cold room, the pH at 0 hour was measured by inserting the pH meter electrode probe between the 12th and 13th ribs, and the carcass was weighed to obtain the hot carcass weight. The carcass was then stored in a cold room at 4°C. After 24 hours, the pH at 24 hours was measured again, and the carcass was weighed to obtain the cold carcass weight. Carcass yields were calculated by comparing the hot carcass weight and cold carcass weight to the animal's weight at slaughter. Cooling losses were determined by the difference between the hot carcass weight and the cold carcass weight. After weighing, the left and right *Longissimus lumborum* (LL) muscles were dissected, packaged, labeled, and stored in a freezer (-20°C) for subsequent evaluation of physicochemical composition and fatty acid profile (FA).

2.3. COMPOSITION AND PHYSICAL-CHEMICAL PROPERTIES OF THE LONGISSIMUS LUMBORUM MUSCLE

Upon receiving the *Longissimus lumborum* samples, a fresh muscle cut was made, allowing it to bloom at temperatures between 6 and 7 °C for 40 minutes for color evaluation (BIFFIN *et al*., 2019), using a Minolta CR-400 colorimeter (Konica Minolta, Tokyo, Japan). The device was calibrated before each analysis using a white tile standard. After exposing the samples to the atmosphere for 30 minutes for myoglobin oxygenation, measurements were taken in triplicate in the CIE (Commission internationale de l'éclairage) system for L* or luminance (L* 0 = black; 100 = white), a* or redness, and b* or yellowness (MILTENBURG *et al*., 1992). The chroma index (C*) was determined from the a* and b* data according to the formula C* = [(a*)^2 + (b*)^2]^0.5 (BOCCARD *et al*., 1981).

For the determination of water-holding capacity (WHC) of the LL muscle, approximately 5.0 g samples were taken, placed between circular paper filters (Albert

238, 12.5 cm in diameter), and subjected to a 10 kg load for 5 minutes (HAMM, 1986). Subsequently, the samples were weighed, and WHC was obtained as the proportion between the weight difference of the samples before and after exposure to the load.

The determination of cooking loss (CL) was performed according to the American Meat Science Association (AMSA, 2015) recommendations, with duplicate assessments on samples free of subcutaneous fat and 2.5 cm in thickness. The meats were pre-weighed and cooked until the geometric center reached 71°C on a grill (George Foreman® Jumbo Grill GBZ6BW, Rio de Janeiro, Brazil) using a stainless steel thermocouple (Gulterm 700; Gulton in Brazil). After cooking, the steaks were cooled and allowed to reach room temperature to stabilize their temperature before being weighed. CL was calculated based on the weight difference of the samples before and after cooking, with values expressed as g/100 g of drip.

From the steaks used for CL evaluation, three central samples measuring approximately 1.0 cm in diameter and 2.0 cm in length, parallel to the muscle fibers, were removed to perform shear force (SF) measurements using a texture analyzer (Texture Analyzer TX-TX2, Mecmesin, Nevada, United States) equipped with a Warner-Bratzler shear blade with a load of 25 kgf and a cutting speed of 20 cm/min. The shear force values obtained were expressed in Newtons (N) according to the standard procedure recommended by the Meat Animal Research Center (SHACKELFORD; WHEELER; KOOHMARAIE, 1999).

For chemical analyses, the meat was lyophilized in advance, and moisture content was evaluated using method 967.03; ash content, method 930.05; ether extract, method 920.39; and crude protein, method 981.10 (AOAC, 2015).

2.4. CHEMICAL ANALYSES OF INGREDIENTS, DIETS, AND LEFTOVERS

At the end of the experiment, the samples of ingredients, diets, and leftovers were thawed and pre-dried at 55 °C for 72 hours, ground using a Willey mill (Marconi, Piracicaba, São Paulo, Brazil) with a 1.0 mm mesh sieve, and sealed in plastic containers for chemical analysis of the content of dry matter (DM; method 934.01; AOAC, 2015), ash (method 930.05; AOAC, 2015), ether extract (EE; Method 920.39; AOAC, 2015), and crude protein (CP; N × 6.25; Kjeldahl method 981.10; AOAC, 2015).

For the determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF), the methodology described by Van Soest *et al*. (1991) with modifications proposed by Senger *et al*. (2008) was used. The NDF content was corrected for ash and protein, where the residue from boiling in neutral detergent was incinerated in a muffle furnace at 600°C for 4 hours. The protein correction was performed by subtracting the insoluble neutral detergent protein (iNDP). The lignin determination was performed according to method 973.18 (AOAC, 2015), using 72% sulfuric acid in the treatment of the ADF residue. The levels of insoluble neutral detergent protein (iNDP) and insoluble acid detergent protein (iADP) were obtained following the recommendations of Licitra *et al*. (1996).

Non-fiber carbohydrates (NFC) were estimated using the equation proposed by Hall (2003): NFC (g DM/kg) = 1000 - [(CP - CPu + U) + NDFap + EE + ash], where CP represents the crude protein content, CPu is the crude protein derived from urea, U is the urea content, NDFap is the NDF adjusted for ash and nitrogen compounds, and EE is the ether extract content. In the calculation, the value of NDF corrected for ash and protein, as described by Mertens (1997), was considered.

Total digestible nutrients (TDN) of the feeds were calculated using the following equation: TDN = CPD + NFCD + NDFapD + 2.25 × EED, from the digestibility trial, where D represents the digestible nutrients.

Metabolizable energy (ME) was calculated according to Weiss (2020), i.e., ME = 0.82 × digestible energy (DE), and DE was obtained from the TDN concentrations of the diet. The total intake of fatty acids (FA) was calculated based on the DM intake and the FA composition of the diet ingredients, assuming that the leftovers had the same FA composition as the consumed feed, according to Barbosa *et al*. (2021).

2.5. FACIDS PROFILE OF INGREDIENTES, DIETS AND MEAT

The fatty acid profiles of the ingredients used in the experimental diets and the meat were also assessed. For the evaluation of the fatty acid profile, methyl esters of fatty acids derived from vegetable fat and encapsulated materials were obtained using the one-step extraction method with 1.25N HCl in methanol, with 19:0 used as an internal standard (SUKHIJA; PALMQUIST, 1988). To convert the samples into methyl esters of fatty acids, a basic catalysis method followed by acid catalysis was employed, as described by Oliveira *et al*. (2016). The analysis of methyl esters of fatty acids was performed using

gas chromatography coupled with flame ionization detection (GC-FID, Shimadzu GC-2010 Plus, Shimadzu Corp., Kyoto, Japan) with a 100% cyanopropyl polysiloxane capillary column (SP 2560; dimensions: 100 m length, 0.25 mm internal diameter, and 0.20 μm film thickness, Supelco Inc., Bellefonte, PA). The identification of fatty acid methyl esters (FAMEs) was done by comparing retention times with authentic standards (37 Component FAME Mix from Supelco Inc.) and published chromatograms (ALVES; BESSA, 2014). Furthermore, the identification of methyl esters of fatty acids, including branched-chain fatty acids (BCFAs), was confirmed by gas chromatography coupled with mass spectrometry (GC-MS) using a gas chromatograph (Shimadzu GC-MS QP 2010 Plus).

The GC-FID analysis was performed with injector and detector temperatures maintained at 220 °C and 250 °C, respectively. The initial oven temperature of 50 °C was held for 1 minute, followed by a temperature increase of 50 °C/min up to 150 °C, where it was held for 20 minutes. Subsequently, the temperature increased at a rate of 1 °C/min to 190 °C and finally increased at a rate of 2 °C/min to 220 °C, where it was held for 30 minutes. Helium was used as the carrier gas with a flow rate of 1 mL/min. For injection, 1 μL of sample (1-2 mg FAME/ml) was used, with a split ratio of 50:1. The GC-MS conditions, including the capillary column and GC settings, were similar to those of GC-FID. The MS conditions included an ion source temperature of 200 °C, an interface temperature of 240 °C, and an electron emission voltage of 70 eV.

2.6. STATISTICAL ANALYSIS

The experimental design followed the premises of a randomized block design, in which each lamb was experimental unit (replication) and two blocks were formed based on the body weight of the animals at the beginning of the experiment. Four treatments were tested: 0.5 inclusion of free urea (U) as control treatment and SRU_{40} encapsulated in low-*trans* vegetable fat at 1.25, 2.0 and 3.0% of total diet DM.

The data obtained were analyzed using the MIXED procedure of SAS 9.4 considering the variables block and block × treatment as random effects according to the following model:

$$Yijk = \mu + \tau i + \beta j + \tau\beta ij + \varepsilon ijk$$

Where: Y_{ijk}= value observed in the experimental unit that received treatment i, replication j; μ= general average common to all observations; τ_i = effect of treatment $_i$; βj

= effect of block j; $\tau\beta_{ij}$ = effect of interaction between treatment i and block j; ε_{ijk} = random error.

The collected data underwent analysis of variance using the PROC MIXED command in SAS. Mean comparisons were conducted through orthogonal contrasts, which were established using the PROC IML. These designated contrasts aimed to independently evaluate the control treatment (free urea U0.5) versus SRU1.25. This comparison was made because they had the same theoretical urea quantity but differed in terms of being free urea versus protected urea. The purpose was to investigate the impact of vegetable fat on urea protection efficiency. Additionally, linear and quadratic contrasts were examined across the three SRU inclusion levels (1.25%, 2%, and 3%). Statistical significance was determined at a threshold of $P < 0.05$.

3. RESULTS

The inclusion of SRU_{40} in the lamb diet did not alter DM intake ($P = 0.924$), resulting in an average daily intake of 1027 g, as well as protein intake, either due to the way urea was provided ($P = 0.674$), whether free or protected, or due to the replacement of soybean meal by the inclusion of protected urea levels in the diet ($P = 0.948$). However, concerning the intakes of EE and ME, there was a linear increase ($P = 0.001$ and $P = 0.023$), following what was offered in the experimental diets (Table 3). Regarding the consumption of fatty acids by the animals (Table 3), the only ones that did not change with the inclusion of SRU_{40} were 20:0 ($P = 0.485$) and 18:3n-6 ($P = 0.893$); for all other fatty acids, there was a linear increase in consumption.

Table 3. Final Weight and Dry Matter Intake, and Fatty Acid Consumption of lambs fed slow-release urea (SRU40) produced from 60% lipid matrix of vegetal fat (VF) and 40% urea (U) as core.

Item	U	SRU_{40} (% DM total)			SEM[2]	*P*-value[3]		
	0.5	1.25	2	3		$U_{0.5}$×$SRU_{1.25}$	Linear	Quadratic
Initial BW (kg)	18.2	17.5	18.2	18.0	-	-	-	-
Slaughter BW (kg)	31.0	29.9	30.5	30.0	1.42	0.598	0.743	0.733
DM intake (g/day)	1025	1028	1028	1027	69.4	0.743	0.852	0.597
CP intake (g/day)	156	154	154	151	10.0	0.674	0.948	0.579
EE intake (g/day)	3.05	3.09	3.15	3.21	0.21	0.826	0.023	0.847
Metabolizable Energy (MJ/kg/day)	2.99	3.03	3.08	3.14	0.21	0.826	0.023	0.847
Microbial protein (g/day)	29.2	29.6	31.1	33.2	2.65	0.899	0.014	0.531
Fatty acids intake (g/day)								
C14:0	0.041	0.048	0.053	0.056	0.003	0.227	0.002	0.913
C16:0	8.52	9.51	10.43	10.77	0.64	0.503	0.021	0.720
C17:0	0.005	0.008	0.010	0.011	0.001	0.001	<0.001	0.957
C18:0	3.02	3.51	3.93	4.14	0.24	0.301	0.002	0.735
C18:1-trans others	0	0.973	1.63	2.31	0.085	<0.001	<0.001	0.994
C18:1-c9	14.9	17.2	19.4	20.6	1.16	0.312	0.001	0.659
C18:1-c11	0.359	0.522	0.638	0.732	0.036	0.007	<0.001	0.859
C18:1-cis others	0	0.482	0.810	1.144	0.042	<0.001	<0.001	0.997
C18:2 others	0	0.342	0.574	0.811	0.030	<0.001	<0.001	0.993
C18:2 n-6	19.6	21.8	23.9	24.7	1.46	0.535	0.024	0.700
C20:0	0.397	0.425	0.458	0.463	0.028	0.799	0.139	0.673
C18:3 n-3	1.77	1.89	1.97	1.94	0.12	0.839	0.452	0.870
C22:0	0.090	0.121	0.139	0.151	0.008	0.025	<0.001	0.896
C24:0	0.084	0.095	0.102	0.104	0.006	0.425	0.038	0.911

[1]SRU_{40} = slow-release urea produced from 60% lipid matrix of low-*trans* vegetal fat (LTVF) and 40% urea (U).
[2]SEM = Standard error of the mean
[3]P-value < 0.05 point to differences between treatments according to orthogonal contrasts: free urea -$U_{0.5}$ × $SRU_{1.25}$; and linear and quadratic regression

The animals fed with the experimental diets did not show any difference in weight gain, with an average final weight of 30.4 kg (P = 0.983), which consequently did not alter carcass characteristics, with an average cold carcass weight of 13.5 kg (P = 0.410), and for carcass yields. The pH at 0 hours and 14 hours was also not influenced, with means of 6.6 and 5.8, respectively (P = 0.059 and P = 0.904), decreasing appropriately (Table 4).

Table 4. Carcass characteristics. of lambs fed slow-release urea (SRU40) produced from 60% lipid matrix of vegetal fat (VF) and 40% urea (U) as core.

Item	U	SRU_{40}[1] (% total DM)			SEM[2]	*P*-value[3]		
	0.5	1.25	2	3		$U_{0.5}$×$SRU_{1.25}$	Linear	Quadratic
Initial BW (kg)	18.2	17.5	18.2	18.0	-	-		
Slaughter BW (kg)	31.0	29.9	30.5	30.0	1.42	0.598	0.996	0.861
Hot carcass weight (kg)	14.5	14.0	14.1	13.1	0.82	0.301	0.860	0.908
Hot carcass yield (%)	46.65	45.34	45.97	43.76	1.00	0.102	0.619	0.955
Cold carcass weight (kg)	13.8	13.7	13.6	12.9	0.77	0.450	0.854	0.968
Cold carcass yield (%)	44.3	44.2	44.3	42.9	0.82	0.295	0.547	0.885
Cooling losses (g)	327	345	393	339	22.8	0.930	0.966	0.811
Initial pH (0h)	6.53	6.55	6.72	6.53	0.05	0.808	0.430	0.069
Final pH (24h)	5.85	5.84	5.88	5.76	0.13	0.940	0.963	0.683
Fat thickness (cm)	1.87	2.04	1.83	1.93	0.19	0.230	0.280	0.434

[1]SRU_{40} = slow-release urea produced from 60% lipid matrix of low-*trans* vegetal fat (LTVF) and 40% urea (U).
[2]SEM = Standard error of the mean
[3]P-value < 0.05 point to differences between treatments according to orthogonal contrasts: free urea -$U_{0.5}$ × $SRU_{1.25}$; and linear and quadratic regression

When we evaluated the proximate composition of the meat, the inclusion of ULL40 did not influence the results. The meat maintained the levels of 75.1% moisture (P = 0.233), 20.7% protein (P = 0.295), 12.5% lipids (P = 0.194), and 1.10% ash (P = 0.353) in its composition. Similarly, meat quality parameters were not altered for color, drip loss, cooking loss, and shear force (Table 5).

Table 5. Chemical composition and meat quality of the Longissimus lumborum muscle in lambs fed slow-release urea (SRU40) produced from 60% lipid matrix of vegetal fat (VF) and 40% urea (U) as core.

Item	U	SRU_{40}[1] (% total DM)			SEM[2]	*P*-value[3]		
	0.5	1.25	2	3		$U_{0.5}$×$SRU_{1.25}$	Linear	Quadratic
Chemical composition (g/100 g meat)								
Moisture	75.1	75.5	75.1	74.8	0.25	0.236	0.205	0.114
Protein	20.7	20.3	20.7	21.1	0.27	0.298	0.213	0.165
Lipid	12.3	12.7	13.0	12.0	0.70	0.133	0.839	0.637
Ash	1.09	1.08	1.12	1.12	0.02	0.869	0.096	0.994
Meat quality								
Color indexes								
Luminosity (L*)	40.4	39.7	40.2	40.6	0.74	0.174	0.253	0.215
Redness (a*)	18.6	18.8	18.7	18.6	0.42	0.650	0.826	0.699
Yellowness (b*)	0.89	0.91	1.09	0.93	0.36	0.534	0.336	0.814
Chroma (C*)	18.6	18.9	18.7	18.6	0.43	0.646	0.843	0.678
WHC (g/100g meat)	25.8	25.7	24.6	25.3	1.42	0.996	0.134	0.843

CWL (g/100g meat)	31.3	31.0	28.1	29.3	3.35	0.957	0.473	0.899
WBSF (N)	13.2	13.0	12.0	14.1	1.61	0.934	0.134	0.843

[1]SRU_{40} = slow-release urea produced from 60% lipid matrix of low-*trans* vegetal fat (LTVF) and 40% urea (U).
[2]SEM = Standard error of the mean
[3]P-value < 0.05 point to differences between treatments according to orthogonal contrasts: free urea -$U_{0.5}$ × $SRU_{1.25}$; and linear and quadratic regression

Table 6. Fatty acids composition (g/100 g FA) of the Longissimus lumborum muscle in lambs fed slow-release urea (SRU40) produced from 60% lipid matrix of vegetal fat (VF) and 40% urea (U) as core.

	U	SRU_{40}[1] (% total DM)			SEM[2]	*P*-value[3]		
Item	0.5	1.25	2	3		$U_{0.5}$×$SRU_{1.25}$	Linear	Quadrático
Saturated fatty acid (SFA)								
10:0	0.115	0.106	0.115	0.102	0.008	0.501	0.686	0.328
12:0	0.075	0.075	0.072	0.074	0.004	0.971	0.860	0.679
14:0	1.792	1.701	1.861	1.805	0.762	0.587	0.575	0.421
15:0	0.210	0.216	0.257	0.241	0.014	0.752	0.251	0.078
16:0	23.52	21.99	22.75	22.40	0.603	0.077	0.671	0.420
17:0	0.701	0.689	0.785	0.704	0.029	0.755	0.874	0.012
18:0	15.66	16.43	16.12	17.18	0.873	0.571	0.554	0.587
20:0	0.045	0.042	0.061	0.057	0.006	0.879	0.417	0.397
22:0	0.002	0.004	0.000	0.000	0.002	0.541	0.175	0.336
Branched chain FA (BCFA)								
iso-14:0	0.027	0.029	0.038	0.032	0.006	0.864	0.052	0.367
iso-15:0	0.062	0.059	0.062	0.062	0.007	0.834	0.002	0.870
anteiso-15:0	0.101	0.100	0.103	0.115	0.014	0.977	0.041	0.869
iso-16:0	0.112	0.133	0.135	0.144	0.018	0.008	0.020	0.930
iso-17:0	0.216	0.225	0.208	0.205	0.013	0.069	0.027	0.629
anteiso-17:0	0.389	0.364	0.387	0.364	0.028	0.614	0.966	0.587
iso-18:0	0.115	0.124	0.123	0.108	0.015	0.726	0.494	0.746
Monounsaturated fatty acid (MUFA)								
c9-14:1	0.062	0.066	0.075	0.066	0.010	0.789	0.938	0.421
c7-16:1	0.229	0.239	0.239	0.252	0.014	0.658	0.523	0.773
c9-16:1	1.528	1.479	1.611	1.431	0.124	0.792	0.734	0.338
c9-17:1	0.450	0.471	0.555	0.453	0.034	0.709	0.626	0.060
c9-18:1	45.53	43.28	44.32	43.20	0.745	0.045	0.864	0.247
c11-19:1	0.048	0.042	0.043	0.031	0.007	0.535	0.301	0.553
c9-19:1	0.082	0.076	0.081	0.071	0.007	0.631	0.670	0.553
Biohidrogenation intermediates (BI)								
c11-18:1	0.978	1.018	1.031	0.979	0.040	0.529	0.509	0.578
c12-18:1	0.098	0.208	0.207	0.234	0.030	0.010	0.027	0.716
c13-18:1	0.062	0.079	0.079	0.074	0.008	0.179	0.675	0.839
c15-18:1	0.034	0.040	0.042	0.042	0.004	0.001	0.848	0.880
c16-18:1	0.025	0.055	0.046	0.045	0.006	<0.001	0.146	0.373
t9-18:1	0.173	0.219	0.221	0.219	0.015	0.039	0.962	0.932
t10-18:1	0.215	0.368	0.343	0.391	0.058	0.050	0.729	0.584
t11-18:1	0.542	0.963	0.793	0.797	0.093	0.001	0.186	0.326

t12-18:1	0.158	0.237	0.195	0.194	0.027	0.057	0.307	0.501
t6/7/8-18:1	0.172	0.234	0.209	0.239	0.018	0.010	0.737	0.170
t16/c14-18:1	0.110	0.165	0.166	0.197	0.018	0.026	0.002	0.601
c9t11-18:2 (CLA)	0.288	0.510	0.401	0.390	0.051	0.003	0.103	0.326
Polyunsaturated fatty acid (PUFA)								
c9t12-18:2	0.028	0.059	0.057	0.058	0.008	0.008	0.932	0.868
c9t13/c9t14/c8t12-18:2	0.099	0.149	0.162	0.171	0.018	0.033	0.340	0.844
t11c15/t10c15-18:2	0.011	0.019	0.021	0.019	0.004	0.199	0.993	0.656
t8c13/c9t15-18:2	0.070	0.103	0.109	0.113	0.010	0.017	0.449	0.865
t9c12-18:2	0.038	0.058	0.052	0.053	0.005	0.002	0.400	0.479
18:3n-3	0.171	0.188	0.205	0.199	0.021	0.499	0.693	0.570
20:5n-3	0.087	0.095	0.095	0.121	0.015	0.727	0.271	0.597
22:5n-3	0.150	0.205	0.195	0.230	0.023	0.115	0.426	0.476
22:6n-3	0.049	0.060	0.044	0.053	0.011	0.398	0.640	0.238
18:2n-6	3.536	4.805	3.713	4.248	0.374	0.013	0.325	0.045
20:2n-6	0.011	0.009	0.014	0.008	0.002	0.714	0.891	0.333
20:3n-6	0.129	0.144	0.115	0.125	0.016	0.537	0.459	0.319
20:3n-9	0.287	0.331	0.240	0.266	0.034	0.353	0.212	0.124
20:4n-6	1.302	1.605	1.150	1.336	0.224	0.175	0.281	0.077
22:4n-6	0.106	0.124	0.091	0.098	0.014	0.383	0.233	0.215

[1]SRU_{40} = slow-release urea produced from 60% lipid matrix of low-*trans* vegetal fat (LTVF) and 40% urea (U).
[2]SEM = Standard error of the mean
[3]P-value < 0.05 point to differences between treatments according to orthogonal contrasts: free urea -$U_{0.5}$ × $SRU_{1.25}$; and linear and quadratic regression

The fatty acid profile (g/100 g of fatty acids) and fatty acid content (mg/100 g of fresh meat) of the LL muscles are presented in Tables 6 and 7, respectively. The inclusion of SRU40 in the diets did not affect most of the fatty acids. However, there were alterations in the concentration of CLA (P = 0.046) and the intermediates of biohydrogenation, the isomers of 18:1, cis-12 (P = 0.027), cis-13 (P = 0.031), and cis-15 (P < 0.001). However, there was no difference in the sum of BI (P = 0.878). For some branched-chain fatty acids, there were also changes with the inclusion of SRU40, resulting in a linear increase in the sum of BCFA content (P = 0.048). The total trans-MUFA increased linearly (P = 0.003), resulting in a linear reduction in the ΣMUFA/ΣSFA ratio (P = 0.028). The total PUFA also increased linearly (P = 0.037), reflecting the linear increase in PUFA from the n-3 family (P = 0.048).

Table 7. Sums of fatty acid groups (mg/100 g of fresh meat), ratios, and health indices of the Longissimus lumborum muscle in lambs fed slow-release urea (SRU40) produced from 60% lipid matrix of vegetal fat (VF) and 40% urea (U) as core.

	U	SRU_{40}[1] (% total DM)			SEM[2]	*P*-value[3]		
Item	0.5	1.25	0.5	1.25		$U_{0.5}$×$SRU_{1.25}$	Linear	Quadrático
Sum (∑)								
Total AG	2063	2014	2207	2070	135	0.474	0.706	0.526
Saturated FA	875	855	951	908	64.0	0.426	0.073	0.388
Total MUFA	1058	1005	1112	1014	74.1	0.620	0.538	0.713
cis-MUFA	1029	958	1070	969	72.7	0.700	0.441	0.755
trans-MUFA	26.3	43.7	38.1	40.3	2.91	0.011	0.003	0.222
Total PUFA	131	154	144	148	6.04	0.059	0.037	0.620
n-3 PUFA	110	129	121	125	5.62	0.207	0.046	0.632
n-6 PUFA	11.0	12.2	13.3	13.6	1.33	0.256	0.613	0.247
Total BCFA	19.9	19.7	22.6	20.6	1.87	0.333	0.048	0.417
Total BI	65.2	64.9	71.7	65.5	6.53	0.505	0.845	0.643
Ratio								
ΣMUFA/ΣSFA	1.20	1.21	1.17	1.13	0.05	0.651	0.028	0.277
ΣPUFA/ΣSFA	0.154	0.189	0.155	0.167	0.01	0.970	0.092	0.433
n–6:n–3	11.3	11.6	9.7	9.5	1.19	0.367	0.952	0.194
Indexes								
Atherogenicity	0.537	0.525	0.536	0.540	0.02	0.978	0.785	0.772
Thrombogenicity	89.7	103	102	105	5.31	0.131	0.160	0.257
h/H index	1.82	1.81	1.81	1.78	0.05	0.909	0.803	0.814
Desirable FA	1498	1474	1610	1514	90.8	0.410	0.738	0.475
Enzymatic activity								
Δ9–desaturase C16	6.16	6.91	6.53	6.09	0.39	0.527	0.417	0.620
Δ9–desaturase C18	75.0	73.9	73.4	71.6	1.38	0.457	0.529	0.241
Elongase	71.0	70.9	71.3	71.5	0.71	0.782	0.987	0.590

[1]SRU_{40} = slow-release urea produced from 60% lipid matrix of low-*trans* vegetal fat (LTVF) and 40% urea (U).
[2]SEM = Standard error of the mean
[3]P-value < 0.05 point to differences between treatments according to orthogonal contrasts: free urea -$U_{0.5}$ × $SRU_{1.25}$; and linear and quadratic regression

4. DISCUSSION

The inclusion of SRU_{40} in the diet did not interfere with animal consumption, which is reinforced by the equality in DM intake, and especially by the consumption of CP and EE, given that the diets were isonitrogenous, and the EE level of the diet increased due to the vegetable fat present in the encapsulate. When considering the individual consumption of fatty acids, we also observe that the effects observed align with the profile of fatty acids present in the ingredients, increasing linearly with their concentration in the diet.

Diets rich in EE can reduce the DM intake of small ruminants when they exceed 50g/kg, especially when there is a higher concentration of monounsaturated fatty acids (MUFAs) (SOUSA *et al*., 2022). The diets with 2 and 3% of SRU40 contained 55.51 and 62.50 g/kg of EE, respectively, and the VF had a total of 32.24 g/100g of MUFA in its composition, which ended up increasing the concentration of MUFA in the diets. These two factors combined could justify a reduction in DM intake; however, this effect was not observed in our study.

Despite the metabolizable energy (ME) content of the diet increasing due to the higher energy content in the fat and ME consumption also increasing linearly, this did not impact the final weight of the animals. This increase in consumption was only 0.15 MJ/kg per day between the treatment without SRU40 and the one with the highest inclusion, which was insufficient to impact the final weight of the animals. Ettoumia *et al*. (2022) conducted a meta-analysis on ME variation in sheep diets and found that an increase of 1 MJ/kg in daily consumption could result in a 0.14 kg increase in slaughter weight, highlighting that significant effects on final weight require a substantial increase in ME intake, which did not occur in the present study.

According to Mohapatra and Shinde (2018), the fat content in the carcass determines the slaughter weight and, consequently, the carcass quality in sheep. Since no differences were found in subcutaneous fat thickness, which is an indicator of fat content in the carcass, and the carcass weight was equal among the tested diets, this could explain the similarity in quality parameters. The different experimental diets also did not influence the carcass pH before and after cooling. This can be attributed to the similar protein levels in the diets and the same feeding and slaughter conditions applied during the experiment (WANG *et al*., 2023). Furthermore, the pH remained within an acceptable range, indicating that muscle transformation into meat during cooling occurred as expected, ensuring meat quality (MANCINI; HUNT, 2005).

Even though the diets had different compositions, especially regarding EE content, this did not affect the chemical composition of the meat or the quality parameters. This demonstrates the potential of using SRU40, as it did not negatively impact these parameters, which is important for enhancing the competitiveness of meat products and ensuring consumer satisfaction (JIA *et al*., 2022).

Analyzing the profile of fatty acids present in the meat of sheep fed SRU_{40}, a significant alteration was observed, mainly in branched-chain fatty acids (BCFA), both individually and in the sum. These fatty acids are primarily derived from microbial

synthesis in the rumen, and previous studies (FIEVEZ et al., 2012) suggest that variations in the concentrations of these fatty acids in ruminant products are related to changes in ruminal microbiota. In the present study, the inclusion of SRU_{40} resulted in a linear increase in microbial protein production, justifying the increasing effect on the concentration of these fatty acids. However, it is worth noting that an increase in BCFA leads to a "goaty" aroma in cooked meat (KHAN; JO; TARIQ, 2015), which could be a positive point for some consumers.

The increase in microbial protein production and branched-chain fatty acids (BCFA) due to the inclusion of SRU_{40} in the animals' diet indicates that the unsaturated fatty acids present in the VF underwent biohydrogenation by ruminal microorganisms, reducing their toxicity. This effect is evidenced by the linear increase in biohydrogenation intermediates, such as the isomers of 18:1 (cis-12, cis-13, and cis-15), as well as the maintenance of the concentration of c9-18:1.

Regarding biohydrogenation intermediates and conjugated linoleic acid (CLA), c9t11-18:2 is the main CLA isomer in ruminant tissues and milk and exerts powerful anticancer effects (WANG AND LEE, 2015). The concentration of c9t11-18:2 in meat is often related to the consumption of 18:2n-6 because its biohydrogenation in the rumen produces small amounts of c9t11-18:2 and larger amounts of t11-18:1, which are then endogenously desaturated back to c9t11-18:2 (CHILLIARD *et al*., 2007). Indeed, in the SRU1.25 and SRU2 diets, the consumption of 18:2n-6 increased, and, in the absence of ruminal biohydrogenation pathways shifted to t10, t11-18:1 increased, leading to an increase in the concentration of c9t11-18:2 in meat.

Furthermore, it is important to highlight that VF contains a significant amount of trans-fatty acids resulting from the hydrogenation process during production. The inclusion of this material as a slow-release urea encapsulant in the animals' diet led to a 53.23% increase in total trans-MUFA deposited in the meat when SRU40 was included at 3%. However, this increase did not affect atherogenicity and thrombogenicity indices, as it was compensated by an increase in total polyunsaturated fatty acids (PUFAs) due to the linear increase in n-3 fatty acids. This expansion of PUFAs also resulted in a reduction in the ΣMUFA/ΣSFA ratio.

These results indicate that the inclusion of SRU_{40} in the animals' diet affects fatty acid metabolism and can modulate both biohydrogenation and fat composition in meat. This information is relevant for understanding the nutritional and health effects associated with the consumption of meat from ruminants fed SRU_{40}.

5. CONCLUSION

Vegetable fat has demonstrated efficacy in protecting urea in the rumen and substituting soybean meal in the diet of sheep, yielding promising results. Its use did not negatively affect meat quality or carcass weight, a critical factor for rural producers' remuneration, indicating its viability as a strategy in meat production. Although it contains trans-fatty acids, ruminal biohydrogenation and the increase in polyunsaturated fatty acids in the meat compensated for any negative impact on atherogenicity and thrombogenicity indices. This approach represents an advantageous alternative to enhance competitiveness and consumer satisfaction without compromising meat quality and composition.

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