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Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2024 018192 1

Dados do Depositante (71)

Depositante 1 de 1

Nome ou Razão Social: UNIVERSIDADE FEDERAL DE CAMPINA GRANDE - PB
Tipo de Pessoa: Pessoa Jurídica
CPF/CNPJ: 05055128000176
Nacionalidade: Brasileira
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Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS

Resumo: A presente invenção engloba a obtenção de sistemas microencapsulados, através da microencapsulação de ureia utilizando gordura vegetal hidrogenada com baixo teor de trans como material encapsulante, pela técnica de Fusão (Melt), possibilitando aplicação das microesferas desenvolvidas na dieta de ruminantes. Esta tecnologia contorna as limitações do uso direto da ureia e permite sua liberação programada, proporcionando proteção frente ao ambiente ruminal e disponibilização controlada no sítio de interesse. Isso resulta na redução dos riscos de intoxicação por ureia e melhora o aproveitamento do nitrogênio pela microbiota ruminal, otimizando o uso da
ureia como fonte de nitrogênio não proteico (NPN). A gordura vegetal hidrogenada constitui uma alternativa eficiente para microencapsular a ureia devido às suas características hidrofóbicas e estabilidade térmica, além de ser um produto acessível e de baixa degradabilidade. As formulações
desenvolvidas melhoram a integridade da ureia durante o armazenamento e facilitam a homogeneização com os demais ingredientes da dieta, viabilizando a aplicação das micropartículas na dieta de ovinos e outros ruminantes. Além disso, essa técnica propicia menor excreção de nitrogênio no ambiente, contribuindo para a sustentabilidade ambiental da produção animal. As micropartículas de ureia encapsuladas com gordura vegetal hidrogenada permitem uma liberação gradual da ureia no rúmen, aumentando a eficiência de produção de proteína microbiana e melhorando o balanço de nitrogênio dos animais, sem comprometer a qualidade dos produtos cárneos.

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Documentos anexados

Tipo Anexo	Nome
GRU	29409162325239213.pdf
Comprovante de pagamento de GRU 200	9213.pdf
Documento de Cessão	Procurac¸a~o PI_UFBA_UFCG UREIA DE LIBERAC¸A~O LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS. assinada.pdf
Declaração de período de graça	Declaração de divulgação anterior não prejudicial.pdf
Resumo	Resumo.pdf
Relatório Descritivo	Relatório descritivo.pdf
Reivindicação	Reivindicações.pdf

Acesso ao Patrimônio Genético

☑ Declaração Negativa de Acesso - Declaro que o objeto do presente pedido de patente de invenção não foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, o acesso foi realizado antes de 30 de junho de 2000, ou não se aplica.

Declaração de Divulgação Anterior Não Prejudicial

☑ Artigo 12 da LPI - Período de Graça.

PETICIONAMENTO ELETRÔNICO

Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 04/09/2024 às 11:41, Petição 870240075678

Declaração de veracidade

☑ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

INSTRUÇÕES:

A data de vencimento não prevalece sobre o prazo legal. O pagamento deve ser efetuado antes do protocolo. Órgãos públicos que utilizam o sistema SIAFI devem utilizar o número da GRU no campo Número de Referência na emissão do pagamento. Serviço: 200-Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

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RUA MAYRINK VEIGA 9 24 ANDAR ED WHITE MARTINS , RIO DE JANEIRO - RJ CEP: 20090910

Agência/Código do Beneficiário
2234-9 / 333028-1

Autenticação Mecânica

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Nome do Beneficiário/CPF/CNPJ
INSTITUTO NACIONAL DA PROPRIEDADE INDUST CPF/CNPJ: 42.521.088/0001-37

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Informações de Responsabilidade do Beneficiário

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__ SIAFI2024-DOCUMENTO-CONSULTA-CONDOC (CONSULTA DOCUMENTO)_____
03/09/24 13:24 USUARIO : ELISABETE SOUSA
DATA EMISSAO : 03Set24 TIPO : 1 - PAGAMENTO NUMERO : 2024GR800312
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CODIGO RECOLHIMENTO : 72200 - 6 COMPETENCIA: AGO24 VENCIMENTO: 02Set24
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(+)JUROS/ENCARGOS :
(+)OUTROS ACRESCIMOS :
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PF1=AJUDA PF3=SAI PF2=DADOS ORC/FIN PF4=ESPELHO PF12=RETORNA

MINISTÉRIO DA EDUCAÇÃO
Universidade Federal da Bahia

PROCURAÇÃO

OUTORGANTE: **UNIVERSIDADE FEDERAL DA BAHIA**, Autarquia Pública Federal, criada pelo Decreto-Lei nº 9.155, de 08 de abril de 1946, CNPJ nº 15.180.714/0001-04, com sede na Rua Augusto Viana, s/n, Palácio da Reitoria, Canela, Salvador-BA, CEP: 40110-909, neste ato representada pelo Coordenador de Inovação da Pró-Reitoria de Pesquisa e Pós-Graduação, **Horácio Nelson Hastenreiter Filho**, brasileiro, portador do CPF nº 013.615.107-81 e matrícula SIAPE nº 1698312.

OUTORGADA: **UNIVERSIDADE FEDERAL DE CAMPINA GRANDE – UFCG**, autarquia federal, vinculada ao Ministério da Educação da República Federativa do Brasil, com sede na Rua Aprígio Veloso, nº 882, Bairro: Universitário - CEP 58428-830, Campina Grande - Paraíba, inscrita no CNPJ sob o nº 05.055.128/0001-76, neste ato representado por seu Magnífico Reitor Professor **ANTÔNIO FERNANDES FILHO**, brasileiro, solteiro, servidor público federal, inscrito no CPF sob o nº 981.448.984-00, residente e domiciliado em Campina Grande, na Rua Rodrigues Alves, nº 796, apto. 101, Bairro Prata, conforme Decreto de 22 de fevereiro de 2021, para o exercício da competência que lhe foi delegada..

PODERES: Constitui e nomeia pelo presente instrumento, seu bastante procurador para requerer e obter, em seu nome, do Governo da República Federativa do Brasil, de acordo com as leis e regulamentos em vigor, junto ao **INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL - INPI**, o pedido de patente de invenção **"UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS",** de propriedade da Universidade Federal da Bahia e Universidade Federal de Campina Grande, de acordo com a Lei de Propriedade Industrial, para o que ficam outorgados à Universidade Federal de Campina Grande amplos e ilimitados poderes para atender todas as formalidades legais e regulamentares relacionadas a este mandato, inclusive, para assinar, depositar, retificar, juntar, retirar, documentos e requerimentos, anotar transferências, alteração de nome, sede, cessões, modificações, apresentar ou recorrer sobre oposição, recurso, revisão administrativa, cumprir ou contestar exigências, requerer prorrogações, acompanhar andamento de processos, obter perante quaisquer autoridades federais, estaduais ou municipais, o que for necessário, finalmente, tudo o que for a bem da fiel execução deste mandato, e tudo o mais que se fizer necessário junto ao referido Órgão.

Campina Grande, 9 de agosto de 2024.

Horácio Nelson Hastenreiter Filho
Coordenador de Inovação da Pró-Reitoria de Pesquisa e Pós-Graduação

DECLARAÇÃO DE DIVULGAÇÃO ANTERIOR NÃO PREJUDICIAL

Declaro, para os devidos fins, que houve divulgação prévia de parte da invenção, porém não de seu teor na íntegra e esta divulgação não excedeu 1 ano do presente pedido de depósito. A divulgação foi feita pela publicação de dois artigos científicos, um na revista Small Ruminant Research intitulado "Effect of dietary inclusion of urea encapsulated in low-trans vegetable fat microspheres on fatty acids intake, carcass traits, quality, and fatty acid composition of lamb meat" publicado em maio de 2024 (link do artigo: https://doi.org/10.1016/j.smallrumres.2024.107289) e outro artigo na revista Animal Feed Science and Technology intitulado "Slow-releasing urea coated with low-trans vegetable lipids: Effects on lamb performance, nutrient digestibility, nitrogen balance, and blood parameters" publicado em fevereiro de 2024 (link do artigo: https://doi.org/10.1016/j.anifeedsci.2024.115925), ambos mencionados nos antecedentes da invenção. A tese de doutorado do discente Pedro Henrique Soares Mazza (PPGZOO/UFBA), sob orientação do prof. Ronaldo Lopes Oliveira e coorientação do prof. Leilson Rocha Bezerra, a qual originou a invenção, foi agraciada pela Sociedade Brasileira de Zootecnia com o prêmio de melhor tese de Doutorado defendida em 2023, apresentada à 58ª Reunião da Sociedade Brasileira de Zootecnia (https://sbz.org.br/reuniao2024/).

Segue em anexo os artigos e a tese de doutorado mencionados anteriormente.

Atenciosamente,

Campina Grande, 28 de agosto de 2024.

Leilson Rocha Bezerra

Small Ruminant Research 236 (2024) 107289

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Effect of dietary inclusion of urea encapsulated in low-*trans* vegetable fat microspheres on fatty acids intake, carcass traits, quality, and fatty acid composition of lamb meat

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A R T I C L E I N F O

Keywords:
Fatty acids
Low-*trans* vegetable fat
Meat
Nitrogen
Slow-release urea

A B S T R A C T

This study evaluated the effect of including slow-release urea (SRU) coated from the low-*trans* vegetable fat (LTFV) microspheres in the diet of lambs on fatty acids intake, carcass traits, meat quality, and fatty acid composition of meat. Thirty-two non-castrated Santa Inês lambs (17.98 ± 2.01 kg of initial weight) were used in a randomized complete block design. Experimental treatments included a control which contained 5 g/kg urea ($U_{0.5\%}$) and three other treatments in which SRU (60% LTFV and 40% urea) was incorporated at 1.25% ($SRU_{1.25\%}$); 2% ($SRU_{2.0\%}$) and 3% ($SRU_{3.0\%}$) of total diet dry matter (DM). The inclusion of SRU in lamb diets increased linearly ($P < 0.05$) the intake of ether extract, metabolizable energy, and fatty acids. Carcass traits and physical-chemical composition of the *Longissimus muscle* were not changed by protected urea addition in lamb diet ($P > 0.05$). The addition of the protected urea in lamb diet linearly increased proportions of CLA and the 18:1 isomer (*cis*-12, *cis*-15, and *cis*-16) compared to $U_{0.5\%}$. There was a linear increase in branched-chain fatty acid (BCFA) content ($P = 0.048$) in *Longissimus muscle* due SRU inclusion. There were a quadratic increases for the sums of *trans*-MUFA ($P = 0.003$) and *n*-6 PUFA ($P = 0.046$) and total *P*UFA ($P = 0.037$) as SRU was added to lambs diet. Adding urea coated into low-*trans* vegetable fat microspheres increased the lamb intake of most FA and energy dietary, however, without affecting carcass traits and meat physicochemical composition. The inclusion of protected urea (SRU) in the lamb diet quadratically increases *trans*-MUFA, *n*-6 PUFA, and total PUFA concentrations in meat, and lambs fed with SRU at level 1.25% in DM total diet presented greater CLA and *trans*-MUFA concentrations compared free urea ($U_{0.5\%}$), which is beneficial from the point of view of the lipid quality of the meat and its relationship with consumer health.

1. Introduction

Partial rumen protection of urea maximizes its efficiency in ruminant feeding by promoting its gradual release in the rumen, minimizing the risk of toxicity, and improving the synchronization of nitrogen release with carbohydrate digestion. Several methods, such as encapsulation, microencapsulation, and emulsion, can be employed for urea protection 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 the synchronization of nitrogen release (Melo et al., 2021). New microencapsulation methods for obtaining rumen-protected urea (i.e., release urea - SRU) have used waxes such as carnauba wax (de Medeiros et al., 2019) and beeswax (Carvalho et al., 2019). Due to their high melting point and stability in the rumen, these waxes are efficient

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0921-4488/© 2024 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

Petição 870240075678, de 04/09/2024, pág. 12/108

in coating urea, but they are also expensive and difficult to process and use on a large scale. Thus, it is necessary to search for other materials that are more accessible for coating urea prior to digestion in the rumen.

In order to manufacture SRU of superior quality, the coating agent must exhibit inertness within the rumen, insolubility in water, and possess hydrophobic properties. Margarine and vegetable shortenings, have been widely adopted in the food industry due to their cost-effectiveness and extended shelf life. They are frequently used in various bakery items, including cakes, bread loaves, and cookies. The conventional production of margarine through the partial hydrogenation of vegetable oils results in the generation of high *trans* fatty acids (TFA), with deleterious effects on consumer health. It is well established that TFA have adverse effects on cholesterol levels, including an increase in LDL cholesterol, a decrease in HDL cholesterol, and an overall increase in the ratio of total cholesterol to HDL cholesterol (Mozaffarian and Clarke, 2009). Then, this has lead to the WHO (2023) recommending that TFA intake should be lower than 1% of total calorie intake. Thus, conventional high-*trans* vegetable structured lipids are replaced by low- or zero-*trans* fatty acid fat products that are produced with alternative methodologies (Temkov and Mureșan, 2021). Therefore, it is necessary to know whether the addition of low-trans fatty acids could cause partial biohydrogenation by rumen microbes so that they have the potential to produce saturated fatty acids (SFA), which will be deposited in lamb meat fat.

In addition to its efficiency as a microencapsulation material for SRU production, according to the results demonstrated by Lucena et al. (2024), low-trans fatty acid vegetable fat (LTVF) also holds promise for enhancing animal meat's fatty acid (FA) composition. The augmentation of dietary PUFA in lambs fed concentrated diets could prove advantageous by reducing palmitic acid (16:0), a significant contributor to hypercholesterolemia in meat, while simultaneously increasing the levels of rumenic acid (*trans*11–18:1) and rumenic acid (*cis*9,*trans*11–18:2) (Wang et al., 2012). These acids are known for their health-promoting properties, including anticarcinogenic effects (Alves et al., 2021; WHO, 2023).

It is essential to note that the elevation of trans11–18:1 and *cis*9, *trans*11–18:2 is contingent upon maintaining normal rumen biohydrogenation pathways. In lambs fed concentrate-based diets, these pathways often shift towards *trans*10–18:1 production, as highlighted by previous studies (Bessa et al., 2015; Alves et al., 2021). Recognizing the favorable attributes of LTVF, such as its easy availability, processing convenience, physicochemical stability, and FA composition, positions it as a promising material for urea coating in the rumen.

Our hypothesis centered on coating urea with LTVF to produce a SRU which would enable lower amounts of soybean meal to be fed in the diet and lead to a controlled release of urea in the rumen. This, in turn, was expected to facilitate improved synchronization of ammoniacal nitrogen in the rumen, all while preserving the integrity of biohydrogenation pathways and the quality of lamb meat, including its fatty acid composition.

Therefore, this study aimed to assess the impact of including LTVF-coated urea for slow release into the rumen in comparison to traditional uncoated urea on fatty acids intake, carcass traits, meat quality, and the profile of fatty acids in lamb meat.

2. Material and methods

2.1. Production of slow-release urea (SRU)

To produce slow-release urea (SRU) into the rumen, a low-*trans* vegetable fat (LTVF) served as the coating material, consisting of vegetable oils (primarily soybean oil), antioxidants (term-butyl-hydroquinone and citric acid), and the antifoaming agent dimethylpolysiloxane, and feed grade urea was used as such core. The chosen LTVF coating, known as Cukin vegetable fat from Bunge Alimentos S.A., exhibited a minimum smoke (burning) point of 225°C. The core-to-wall material ratio for urea and LTVF was 40:60, respectively (SRU). Employing the Fusion-Emulsification technique outlined by De Medeiros et al. (2019), the encapsulation process utilized soy lecithin as the emulsifying agent at a ratio of 1% relative to the mass of LTVF.

The LTVF was precisely weighed on an analytical balance, transferred to a beaker, and then combined with a surfactant (40% soy lecithin) at a 1% ratio of the vegetable fat's mass. This mixture was maintained in a thermostatic bath at 60°C. Simultaneously, in another beaker, urea was dissolved in distilled water to create a 50% (w/w) and a solution with a pH of 9.2. This urea solution was also placed in the thermostatic bath to aid dissolution and equalize temperatures with the LTVF. Once the materials reached a stable temperature, the urea solution was gradually introduced into the beaker containing LTVF and soy lecithin, while mixing with a homogenizer (T25 digital Ultra-Turrax®, Ika, USA). Subsequently, the emulsion was transferred to plastic containers and subjected to a forced air circulation oven at 55°C for 24 hours to facilitate dehydration. After drying, the material was removed from the oven, allowed to cool to room temperature, and then stored in a refrigerator at 2°C for subsequent analysis and application (Lucena et al., 2024).

2.2. Ethical considerations, animals, management, diets, experimental design, intake

All animal care and management practices were conducted exclusively after receiving approval (Protocol number 58/2021) and in strict adherence to the recommendations of the Ethics Committee on Animal Use (CEUA) of the Federal University of Campina Grande, Paraiba, Brazil.

Thirty-two non-castrated Santa Inês lambs, initially averaging six months in age and 18.0 ± 2.01 kg in BW were grouped in a randomized complete block (02) design, with four treatments and eight replications. The control treatment contained 5 g/kg uncoated urea ($U_{0.5\%}$), and with 3 other treatments in which SRU was added to the diets at proportions of 1.25% ($SRU_{1.25\%}$), 2.0% ($SRU_{2.0\%}$), and 3% ($SRU_{3.0\%}$) on a total diet dry matter (DM) basis.

Lambs were initially weighed in a fasting state, identified, vaccinated against clostridiosis (Biovet Resguard Multi®, São Paulo, Brazil), orally dewormed with a 5% Levamisol hydrochloride-based dewormer (dosage 1.0 mL/10 kg BW; Ripercol® L, São Paulo, Brazil), and supplemented with a vitamin mix (A, D, and E). The animals were housed individually in wooden pens suspended 70 cm above the ground. Each pen was equipped with a drinking trough and a feeder. The experiment lasted for 75 days, with an initial 15-day period for adaptation followed by 60 days for performance evaluation.

Diets were formulated to meet the requirements of growing male lambs for an average daily gain of 200 g/day, following NRC (2007) guidelines. The diets had a forage-to-concentrate ratio of 30:70, with Tifton-85 hay (*Cynodon sp*.) as the forage source, and the concentrate composed of ground corn, corn grain silage hydrated by cactus mucilage, soybean meal, mineral mixture, and uncoated urea or SRU (Tables 1 and 2). To serve as a soluble carbohydrate source and enhance urea utilization in the rumen, corn grain silage moistened with cactus (*Opuntia stricta* Haw.) forage mucilage was added and mixed at a 75:25 ratio. Uncoated urea and SRU were incorporated into the concentrate and homogenized in a mixing wagon Y-type (Coppi®, Santa Catarina, Brazil).

A gradual adaptation to urea was implemented, introducing it during the adaptation period. The diet was offered as a total mixed ration (TMR) in two equal portions at 0700 h and 1700 h, and was adjusted daily based on the weighing of refusals from the previous day to ensure between 10% and 20% of refusals. Water was provided *ad libitum*. Refusal samples were collected every three days, composite samples were analyzed to determine DM intake. 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 refusals had the same FA

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Table 1
Chemical composition and fatty acid (FA) profile for feed ingredients used in experimental diets (DM basis).

Item (g/kg DM)	Ground corn silage[a]	Urea	SRU[b]	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
$_{a}$NDF[c]	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/100 g FA)						
14:0	0.00	-	0.08	0.00	0.00	1.09
16:0	18.4	-	12.0	18.1	16.0	32.9
16:1*c*9	0.00	-	0.10	0.00	0.00	0.00
17:0	0.53	-	0.04	0.00	0.00	0.00
18:0	5.62	-	5.93	7.38	5.84	9.48
18:1-*trans*	0.00	-	11.9	0.00	0.00	0.00
18:1-*c*9	34.1	-	29.1	16.2	33.2	9.22
18:1-*c*11	1.05	-	1.99	1.66	0.66	0.90
Other-C18:1-*cis*	0.00	-	5.88	0.00	0.00	0.00
Other-18:2	0.00	-	4.18	0.00	0.00	0.00
18:2 *n*-6	38.6	-	26.7	50.8	42.1	12.6
20:0	0.95	-	0.34	0.37	0.73	2.01
18:3 *n*-3	0.81	-	1.26	4.90	1.48	27.5
22:0	0.00	-	0.37	0.59	0	2.01
24:0	0.00	-	0.12	0.00	0.00	2.25
∑SFA	25.5	-	18.9	26.4	22.6	49.8
∑MUFA	35.1	-	48.9	17.9	33.9	10.1
∑PUFA	39.4	-	32.2	55.7	43.5	40.1

[a] Hydrated with cactus pear mucilage;
[b] SRU = slow-release urea produced from 60% lipid matrix of low-*trans* vegetable fat (LTVF) and 40% urea (U);
[c] Neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash;

composition as the consumed feed, according to Barbosa et al. (2021).

2.3. Intake, slaughter, and carcass traits

Daily, before morning feeding, individual intakes were measured according to the refusal left by each animal. Weekly, lambs were weighed before morning feeding and samples of each experimental diets were collected. At the end of the 60 experimental days, after 12 hours of solid fast, lambs were again weighed before slaughter. Then, lambs were transported in an appropriate truck with minimal stress to a slaughterhouse at 3.2 km from the experimentation farm and were slaughtered on the same day. During slaughter, the lambs 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 cooler, the pH at 0 h 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 h, the carcasses were 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. Determination of drip loss involved extracting *Longissimus lumborum* fresh sample cores with a specialized circular knife, from the right half of the carcass, each having a diameter of 2.5 cm. Before placement in individual tubes, the initial weight of the samples was recorded. Subsequently, the samples were refrigerated at 3–4 ∘C for 48 h. After this period, each core was reweighed, and the drip loss was quantified as % of the weight change during this timeframe (Holman et al., 2020).

The *Longissimus lumborum* was removed from the left side of the carcass and separated from the bone. It was then divided into subsamples, packaged, labeled, and stored in a freezer (–20°C) until subsequent FA determination and chemical and physical analyses.

2.4. Physical-chemical composition of the Longissimus lumborum muscle

Carcass pH was determined before carcass chilling (within 30 min postmortem; 0 h) and 24 h after slaughter, following the methodology of Biffin et al. (2019). Calibration of the pH meter was conducted using pH 4 and 7 buffer solutions, and the temperature during calibration was maintained at 20 °C and monitored with a digital skewer-type probe (Digimed, 300 M, São Paulo, Brazil).

Upon collection, a fresh cross-section of the *Longissimus lumborum* muscle underwent color analysis, allowing it to develop at temperatures between 6 and 7 °C for 40 min., following the protocol of Biffin et al. (2019). The colorimeter used for these measurements was a Minolta CR-400 (8 mm aperture; 10° observer) equipped with a pulsed xenon lamp as its light source and a glass cover on the aperture port. Measurements were conducted using illuminant D65, and the colorimeter was recalibrated before each analysis using a white tile standard.

After exposing the samples to the atmosphere for 30 minutes to allow myoglobin oxygenation, triplicate measurements for L^*, a^*, and b^* values related to color were taken according to the CIE system (Commission Internationale de L'éclairage). The system was calibrated using the CIELAB system with a blank tile. L^* (luminosity), a^* (redness), and b^* (yellowness) indexes were evaluated as per the method outlined by Miltenburg et al. (1992). The saturation index (chroma, C*) was calculated based on the a^* and b^* values using the formula C* = [(*a*)^2 + (*b*)^2] ^0.5, as proposed by Boccard et al. (1981).

For water-holding capacity (WHC) determination, *Longissimus lumborum* muscles, 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 calculated as the proportion between the weight difference of the samples before and after exposure to the load.

Cooking weight loss (CWL) was performed according to the American Meat Science Association (AMSA, 2016) on samples free of subcutaneous fat and 2.5 cm in thickness. The pre-weighed meats were cooked

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Table 2
Ingredient proportions, chemical composition, and fatty acid profile for experimental diets including slow-release urea (SRU) produced from the lipid matrix of low-*trans* vegetable fat.

Item	Urea (% DM total)	SRU[1] (% DM total)		
	$U_{0.5\%}$	$SRU_{1.25\%}$	$SRU_{2.0\%}$	$SRU_{3.0\%}$
Ingredients				
Tifton–85 hay	300	300	300	300
Ground corn	505	498	518	539
Ground corn silage[b]	20	20	20	20
Soybean meal	140	139.5	112	81
Uncoated urea (U)	5.0	-	-	-
Slow-release urea (SRU)[a]	-	12.5	20	30
Mineral mixture[c]	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
$_{a}$Neutral detergent fiber[d]	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[e]	312	310	312	314
Acid detergent insoluble protein[e]	194	192	196	199
Total digestible nutrients	806	814	829	846
Metabolizable energy, MJ/kg[f]	2.98	3.01	3.06	3.12
Fatty acid composition (g/100 g FA)				
14:0	0.084	0.085	0.083	0.082
16:0	17.46	16.70	16.30	15.84
17:0	0.010	0.013	0.015	0.017
18:0	6.196	6.162	6.124	6.090
18:1-*trans*	0.000	1.707	2.549	3.395
c9–18:1	30.46	30.24	30.33	30.39
c11–18:1	0.735	0.916	0.996	1.077
18:1-*cis*	0.000	0.847	1.264	1.683
18:2	0.000	0.600	0.896	1.193
18:2 *n*-6	40.25	38.30	37.31	36.32
20:0	0.814	0.747	0.714	0.681
18:3 *n*-3	3.634	3.316	3.078	2.852
22:0	0.184	0.212	0.216	0.223
24:0	0.172	0.167	0.160	0.153
∑SFA	24.92	24.08	23.58	23.0
∑MUFA	31.20	33.71	35.13	36.541
∑PUFA	43.88	42.21	41.29	40.37

[a] SRU = slow-release urea produced from 60% lipid matrix of low-*trans* vegetable fat (LTVF) and 40% urea; $U_{0.5\%}$ = 5% uncoated urea;
[b] Ground corn silage hydrated with cactus pear mucilage;
[c] 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; 1800 mg of iron; 80 mg of iodine; 1300 mg of manganese; 15 mg of selenium; 3800 mg of zinc; 300 mg of molybdenum; maximum 870 mg of fluoride;
[d] NDF assayed with a heat stable amylase and expressed exclusive of residual ash;
[e] g/kg CP;
[f] Calculated according to Weiss (2020).

until the geometric center reached 71 °C on a grill (George Foreman®, Rio de Janeiro, Brazil), monitored by a stainless-steel thermocouple (Gulterm 700; Gulton in Brazil). After cooking, the steaks were cooled to room temperature before being weighed. CWL was calculated based on the samples' weight difference before and after cooking, expressed as g/100 g drip. Subsequently, three round cores were extracted, parallel to the long axis of the muscle fibers, to perform shear force (SF) determination. Instrumental texture measurements (kgf) were assessed using a texture analyzer (TA-XT /Express enhanced, Hamilton, MA, USA) equipped with a Warner-Bratzler shear blade, a load of 25 kgf, and a cutting speed of 20 cm/min. The SF values obtained were expressed in Newtons (N), following the standard procedure recommended by the Meat Animal Research Center (Shackelford et al., 1999).

The meat was lyophilized for chemical analyses with moisture content determined using method 967.03; ash content determined using method 930.05; ether extract content determined using method 920.39; and crude protein content determined using method 981.10 (AOAC, 2015).

2.5. Chemical analyses of ingredients, diets, and leftovers

Upon completion of the experiment, stored feed ingredients, diet, and refusals samples were thawed and pre-dried at 55 °C for 72 h. Subsequently, they were ground using a Willey mill (Marconi, Piracicaba, São Paulo, Brazil) with a 1.0 mm mesh sieve and then sealed in plastic containers for chemical analysis 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).

The NDF was determined according to Van Soest et al. (1991) with modifications for nonwoven tissue (Senger et al., 2008), using thermostable amylase (Sigma A3306; Sigma–Aldrich, Steinheim, Germany) and expressed exclusive of residual ash ($_{a}$NDF). The lignin determination followed method 973.18 (AOAC, 2015), utilizing 72% sulfuric acid to treat the ADF residue. The levels of insoluble neutral detergent protein and insoluble acid detergent protein were determined following the recommendations of Licitra et al. (1996).

Non-fiber carbohydrates (NFC) were estimated from the equation proposed by Hall (2003): NFC (g DM/kg) = 1000 – [(CP – CPu + U) + aNDF + EE + ash], where CP represents the crude protein content, CPu is the crude protein derived from urea, U is the urea content, apNDF 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, as described by Mertens (1997), was considered. Metabolizable energy (ME) was calculated according to Weiss (2020): ME = 0.82 × digestible energy (DE), and DE was obtained from the results of the following calculation: DE = TDN (%) x 2.25 Kcal.

2.6. Fatty acid profile for feed ingredients, diets, and Longissimus lumborum

The fatty acid (FA) composition of diets and *Longissimus lumborum*, as well as the methyl esters of FA (FAME) derived from LTVF and SRU, were obtained using a one-step extraction method with 1.25 N HCl in methanol, utilizing 19:0 as an internal standard (Sukhija and Palmquist, 1988). To convert the samples into fatty acid methyl esters (FAMEs), a two-step process involving catalysis followed by acid catalysis was employed, following the methodology outlined by Oliveira et al. (2016). The analysis of FAMEs was conducted using GC coupled (GC-FID, Shimadzu GC-2010 Plus, Shimadzu Corp., Kyoto, Japan) equipped 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), with flame ionization detection.

Identification of FAMEs was accomplished by comparing retention times with authentic standards (37 Component FAME Mix from Supelco Inc.) and referencing published chromatograms (Alves and Bessa, 2014; Alves et al., 2021). Additionally, the identification of FAME, including branched-chain fatty acids (BCFA), was confirmed through gas chromatography coupled with mass spectrometry (GC-MS) using a gas chromatograph (Shimadzu GC-MS QP 2010 Plus).

During GC-FID analysis, injector and detector temperatures were maintained at 220 °C and 250 °C, respectively. The initial oven temperature was 50 °C to hold 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 2 °C/min to 220 °C, and it was held for

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30 minutes. Helium gas was the carrier (flow rate of 1 mL/min). For injection, 1 μL of sample (1–2 mg FAME/mL) was used, with a split ratio 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.

Using FA composition data, the sums (Σ) of different FA classification groups were calculated, including saturated fatty acids (ΣSFA), monounsaturated fatty acids (ΣMUFA), polyunsaturated fatty acids (ΣPUFA), BI, *cis*-MUFA, *trans*-MUFA, Total PUFA, *n*-6 PUFA and *n*-3 PUFA, as well as the ratios between PUFA (P) and SFA (S) (P/S = (18:2 *n*–6 + 18:3 *n*–3)/ (12:0 + 14:0 + 16:0), and the ratio Σ*n*–6:Σ*n*–3, based on the identified FA profiles (mg/100 g tissue) to compare with Human nutritional guidelines.

The hypocholesterolemic and hypercholesterolemic (h:H ratio) fatty acid ratio was also determined using the formula proposed by Santos-Silva et al. (2002) along with the determination of desirable fatty acids (DFAs) following the methods outlined by Rhee (1992).

The Δ9-desaturase activity indices by examining the ratios between product and substrate pairs: Δ9-C16, utilizing the *c*9–16:1 and 16:0 pair; Δ9-C18, employing the *c*9–18:1 and 18:0 pair, and elongase activity was estimated according to the methodology of Smet et al. (2004). Additionally, stearoyl-CoA desaturase (SCD) activity indices were estimated by computing the ratio of product/ (substrate + product): SCDi17 = (17:1/(17:1+17:0)100) (Alves et al., 2015).

2.7. Statistical analysis

The experimental design followed the premises of a randomized block design, in which each lamb was an experimental unit (replication), and two blocks were formed based on the lambs' body weight at the experiment's beginning. Block one had an average initial body weight (BW) of 16.7 ± 1.27 kg, and animal block two had a BW average of 19.6 ± 1.13 kg. Each block corresponded to a specific weight range, and the number of animals per block was equally distributed for each treatment, ensuring equitable representation in all weight categories.

Four treatments were tested: 0.5% inclusion of uncoated urea ($U_{0.5\%}$) as control treatment and SRU 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:

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

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 (uncoated urea or $U_{0.5\%}$) versus $SRU_{1.25\%}$. This comparison was made because these diets had the same theoretical urea quantity but differed in terms of being uncoated urea ($U_{0.5\%}$) versus protected urea ($SRU_{1.25}$). 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 intakes of EE ($P = 0.001$), total FA ($P = 0.038$), and ME ($P = 0.023$) linearly increased due to SRU inclusion in lamb diets (Table 3). Intakes of most individual FA linearly increased due to SRU inclusion ($P < 0.05$) with the exception of the ingestion of 20:0 and 18:3*n*-6 ($P > 0.05$).

Lambs fed SRU did not show a difference in slaughter weight, with a mean 30.4 kg ($P = 0.983$), and carcass traits (Table 4) also were not affected by the inclusion of SRU ($P > 0.05$). Meat pH values at 0 and 24 h were also not influenced ($P > 0.05$) by SRU inclusion (Table 4). The centesimal composition of the meat was not affected by SRU inclusion and presented an average of 75.1% moisture, 20.7% protein, 3.0% lipids, and 1.10% ash. Similarly, lamb meat quality parameters (color, WHC, CWL, and WBSF) were not altered ($P > 0.05$) by SRU dietary inclusion (Table 5).

The effects of SRU inclusion on the FA profile (g/100 g of fatty acids) and FA content (mg/100 g of fresh meat) for *Longissimus* muscles are presented in Tables 6 and 7, respectively. Most individual FA was unaffected ($P > 0.05$) by the inclusion of SRU in the diets except for some biohydrogenation intermediates that were increased and minor changes on BCFA (Table 6). FA *c*9*t*11–18:2 (CLA; $P = 0.046$) and most of the

Table 3
Ether extract, metabolizable energy and fatty acids intake of lambs fed slow-release urea (SRU) produced from lipid matrix of low-*trans* vegetable fat (LTVF).

Variables	Inclusion (% DM total)				SEM[2]	*P*-value[3]		
	$U_{0.5\%}$	$SRU_{1.25\%}$	$SRU_{2.0\%}$	$SRU_{3.0\%}$		$U_5 \times SRU_{1.25}$	Linear	Quadratic
Ether extract intake (g/d)	48.8	57.0	64.1	71.0	3.84	0.826	0.023	0.847
Metabolizable energy (MJ/d)	12.5	12.7	12.9	13.2	0.89	0.826	0.023	0.847
Fatty acids intake (g/d)								
14:0	0.020	0.023	0.024	0.028	0.003	0.227	0.002	0.913
16:0	4.10	4.56	4.77	5.34	0.64	0.503	0.021	0.720
17:0	0.002	0.004	0.004	0.006	0.0005	0.001	<0.001	0.957
18:0	1.45	1.68	1.80	2.05	0.181	0.301	0.002	0.735
18:1-*trans*	0.000	0.466	0.747	1.144	0.106	<0.001	<0.001	0.994
c9–18:1	7.15	8.26	8.89	10.24	0.891	0.312	0.001	0.659
c11–18:1	0.173	0.250	0.292	0.363	0.029	0.007	<0.001	0.859
Others-18:1-*cis*	0.000	0.231	0.370	0.567	0.052	<0.001	<0.001	0.997
Others-18:2	0.000	0.164	0.263	0.402	0.037	<0.001	<0.001	0.993
18:2*n*-6	9.45	10.5	10.9	12.2	1.12	0.535	0.024	0.700
20:0	0.191	0.204	0.209	0.230	0.02	0.799	0.139	0.673
18:3*n*-3	0.853	0.905	0.902	0.961	0.09	0.839	0.452	0.870
22:0	0.043	0.058	0.063	0.075	0.006	0.025	<0.001	0.896
24:0	0.040	0.046	0.047	0.052	0.005	0.425	0.038	0.911
Total FA	23.5	27.3	29.3	33.7	2.94	0.04	0.038	0.986

[1]SRU = slow-release urea produced from 60% lipid matrix of low-*trans* vegetable fat (LTVF) and 40% urea; $U_{0.5\%}$ = 5% uncoated urea;
[2]SEM = Standard error of the mean;
[3]*P*-value < 0.05 point to differences between treatments according to orthogonal contrasts: $U_{0.5\%} \times SRU_{1.25\%}$; and linear and quadratic regression for SRU 1.25; 2.0 and 3.0%.

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Table 4
Carcass traits of lambs (n = 32) fed slow-release urea (SRU) produced from lipid matrix of low-*trans* vegetable fat (LTVF).

Variables	Inclusion (% DM total)				SEM[2]	*P*-value[3]		
	$U_{0.5\%}$	$SRU_{1.25\%}$	$SRU_{2.0\%}$	$SRU_{3.0\%}$		$U_5 \times SRU_{1.25}$	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 (%)	46.6	45.3	46.0	43.8	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
Drip loss (%)	3.34	3.45	3.33	3.45	0.05	0.930	0.966	0.811
Initial pH (0 h)	6.53	6.55	6.72	6.53	0.05	0.808	0.430	0.069
Final pH (24 h)	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 = slow-release urea produced from 60% lipid matrix of low-*trans* vegetable fat (LTVF) and 40% urea; $U_{0.5\%}$ = 5% uncoated urea;
[2]SEM = Standard error of the mean;
[3]*P*-value < 0.05 point to differences between treatments according to orthogonal contrasts: $U_{0.5\%} \times SRU_{1.25\%}$; and linear and quadratic regression for SRU 1.25; 2.0 and 3.0%.

Table 5
Chemical composition and meat quality of the *Longissimus lumborum* muscle in lambs fed slow-release urea (SRU) produced from lipid matrix of low-*trans* vegetable fat (LTVF).

Variables	Inclusion (% DM total)				SEM[2]	*P*-value[3]		
	$U_{0.5\%}$	$SRU_{1.25\%}$	$SRU_{2.0\%}$	$SRU_{3.0\%}$		$U_5 \times SRU_{1.25}$	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	3.11	3.12	3.08	2.98	0.06	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/100 g meat)	25.8	25.7	24.6	25.3	1.42	0.996	0.134	0.843
CWL (g/100 g 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 = slow-release urea produced from 60% lipid matrix of low-*trans* vegetable fat (LTVF) and 40% urea; $U_{0.5\%}$ = 5% uncoated urea;
[2]SEM = Standard error of the mean;
[3]*P*-value < 0.05 point to differences between treatments according to orthogonal contrasts: $U_{0.5\%} \times SRU_{1.25\%}$; and linear and quadratic regression for SRU 1.25; 2.0 and 3.0%;
[4]Water-holding capacity (WHC); Cooking weight losses (CWL); Warner Bratzler shear force (WBSF).

other biohydrogenation intermediates including the *t*9–18:1, *t*10–18:1, and *t*11–18:1 were increased ($P < 0.05$) by SRU inclusion.

When evaluating the sums of certain fatty acids expressed in mg/100 g of fresh meat (Table 7), there were a quadratic increases for the sums of *trans*-MUFA ($P = 0.003$) and *n*-6 PUFA ($P = 0.046$) and total PUFA ($P = 0.037$) as SRU was added to diets. Health indices and the evaluated enzymatic activities were not affected ($P > 0.05$) by the SRU addition to the diets.

4. Discussion

Diets rich in EE can reduce DM intakes for small ruminants when they exceed 50 g/kg DM, especially when there is a higher concentration of UFA (Sousa et al., 2022). The diets with 20 and 30 g/kg of SRU contained 55.5 and 62.5 g/kg of EE, respectively, and the LTVF had a total of 32.2 g/100 g of MUFA in its composition (Table 1), which ended up increasing the concentration of MUFA in SRU diets.

The LTVF is produced through the hydrogenation of soybean oil and has a low *trans*-fat concentration. However, due to the SRU production process, high-temperature agitation, lecithin, and a urea solution with a pH of 9.2 caused the formation of *trans*18:1 (Carvalho et al., 2019; de Medeiros et al., 2019). Nevertheless, SRU diets still maintained a high concentration of ΣMUFA + ΣPUFA (81.2 mg/100 g FA; Supplementary table), and even so, its inclusion in the diet did not alter DM intake (Lucena et al., 2024).

Lucena et al. (2024) observed that LTVF demonstrated high efficiency in coating urea, exhibiting high yield and coating efficiency. The authors recommended including 3% urea coated by LTVF in the diet of lambs because it improved the CP digestibility and the utilization of ammonia by rumen microorganisms, reducing N-excretion and improving N-balance and microbial protein production without affecting growth.

Despite dietary metabolizable energy (ME) content increasing in SRU diets due to the high energy content in LTVF along with linear increases in ME intake, there were no diet effects on the growth for lambs in the study (Lucena et al., 2024). This increase in ME intake was only 0.7 MJ/kg per day between treatment $U_{0.5\%}$ and the highest inclusion ($SRU_{3.0\%}$), which was insufficient to impact the performance and consequently carcass traits for the lambs (Lucena et al., 2024).

Ettoumia et al. (2022) conducted a meta-analysis on ME density variation in sheep diets and found that an increase of 1.0 MJ/kg DM in daily intake 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 carcass fat content determines the slaughter weight and, consequently, the carcass quality

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Table 6
Fatty acids composition (mg/100 g of muscle) in *Longissimus lumborum* of lambs fed slow-release urea (SRU) produced from lipid matrix of low-*trans* vegetable fat (LTVF).

Fatty acids composition (g/100 g muscle)	Inclusion (% DM total)				SEM[2]	*P*-value[3]		
	$U_{0.5\%}$	$SRU_{1.25\%}$	$SRU_{2.0\%}$	$SRU_{3.0\%}$		$U_{0.5}\times SRU_{1.25}$	Linear	Quadratic
Saturated fatty acids (SFA)								
10:0	1.03	0.81	1.32	1.24	0.01	0.501	0.686	0.328
12:0	1.65	1.61	1.54	1.45	0.01	0.971	0.860	0.679
14:0	2.48	2.22	2.65	2.07	0.01	0.587	0.575	0.421
15:0	4.33	4.43	5.74	4.97	0.02	0.752	0.251	0.078
16:0	14.44	13.90	17.4	14.49	0.04	0.077	0.671	0.420
17:0	36.93	34.24	41.0	37.47	1.03	0.755	0.874	0.012
18:0	323.9	330	355	356	1.18	0.571	0.554	0.587
20:0	484.8	443.1	503	463.7	0.81	0.879	0.417	0.397
Branched chain FA (BCFA)								
iso-14:0	0.619	0.604	0.88	0.621	0.008	0.864	0.052	0.367
iso-15:0	1.238	1.208	1.32	1.242	0.009	0.834	0.002	0.870
anteiso-15:0	2.063	2.014	2.21	2.484	0.019	0.977	0.041	0.869
iso-16:0	2.269	2.618	3.09	2.898	0.024	0.008	0.020	0.930
iso-17:0	4.539	4.632	4.63	4.347	0.018	0.069	0.027	0.629
anteiso-17:0	8.046	7.250	8.61	7.452	0.038	0.614	0.966	0.587
iso-18:0	2.476	2.417	2.65	2.277	0.020	0.726	0.494	0.746
cis-MUFA								
*c*9–14:1	1.24	1.41	1.77	1.45	0.014	0.789	0.938	0.421
*c*7–16:1	4.74	4.83	5.30	5.18	0.019	0.658	0.523	0.773
*c*9–16:1	31.56	29.81	35.53	29.60	0.167	0.792	0.734	0.338
*c*9–17:1	9.28	9.47	12.36	9.32	0.046	0.709	0.626	0.060
*c*9–18:1	939	872	978	894	1.006	0.045	0.864	0.247
*c*11–18:1	20.22	20.54	22.73	20.29	0.054	0.529	0.509	0.578
*c*12–18:1	2.06	4.23	4.63	4.76	0.041	0.010	0.027	0.716
*c*13–18:1	1.28	1.59	1.74	1.53	0.011	0.179	0.675	0.839
*c*15–18:1	0.70	0.81	0.93	0.87	0.005	0.001	0.848	0.880
*c*16–18:1	0.52	1.11	1.02	0.93	0.008	<0.001	0.146	0.373
*c*9–19:1	1.65	1.61	1.77	1.45	0.009	0.631	0.670	0.553
*c*11–19:1	1.03	0.81	0.88	0.62	0.009	0.535	0.301	0.553
cis-PUFA								
18:3*n*-3	3.51	3.83	4.63	4.14	0.028	0.499	0.693	0.570
20:5*n*-3	1.86	2.01	2.21	2.48	0.020	0.727	0.271	0.597
22:5*n*-3	3.09	4.23	4.41	4.76	0.031	0.115	0.426	0.476
22:6*n*-3	1.03	1.21	0.88	1.04	0.015	0.398	0.640	0.238
18:2*n*-6	73.03	96.87	81.88	87.98	0.505	0.013	0.325	0.045
20:2*n*-6	0.21	0.20	0.22	0.21	0.003	0.714	0.891	0.333
20:3*n*-6	2.68	2.82	2.65	2.69	0.022	0.537	0.459	0.319
20:3*n*-9	5.98	6.65	5.30	5.59	0.046	0.353	0.212	0.124
20:4*n*-6	26.82	32.43	25.38	27.74	0.302	0.175	0.281	0.077
22:4*n*-6	2.27	2.42	1.99	2.07	0.019	0.383	0.233	0.215
Trans-Biohydrogenation intermediates (BI)								
*t*6-/*t*7-/*t*8–18:1	3.51	4.63	4.63	4.97	0.02	0.010	0.737	0.170
*t*9–18:1	3.51	4.43	4.86	4.55	0.02	0.039	0.962	0.932
*t*10–18:1	4.54	7.45	7.50	8.07	0.08	0.050	0.729	0.584
*t*11–18:1	11.14	19.33	17.44	16.56	0.13	0.001	0.186	0.326
*t*12–18:1	3.30	4.83	4.41	3.93	0.04	0.057	0.307	0.501
*t*16-/*c*14–18:1	2.27	3.42	3.75	4.14	0.02	0.026	0.002	0.601
*c*9*t*11–18:2 (CLA)	5.98	10.27	8.83	8.07	0.07	0.003	0.103	0.326
*c*9*t*12–18:2	0.62	1.21	1.32	1.24	0.01	0.008	0.932	0.868
*c*9*t*13-/*c*9*t*14-/ *c*8*t*12–18:2	2.06	3.02	3.53	3.52	0.02	0.033	0.340	0.844
*t*11*c*15-/*t*10*c*15–18:2	0.00	0.00	0.00	0.00	0.00	0.199	0.993	0.656
*t*8*c*13-/*c*9*t*15–18:2	0.21	0.40	0.44	0.41	0.01	0.017	0.449	0.865
*t*9*c*12–18:2	1.44	2.01	2.43	2.28	0.01	0.002	0.400	0.479

[1]SRU = slow-release urea produced from 60% lipid matrix of low-*trans* vegetable fat (LTVF) and 40% urea; $U_{0.5\%}$ = 5% uncoated urea;
[2]SEM = Standard error of the mean;
[3]*P*-value < 0.05 point to differences between treatments according to orthogonal contrasts: $U_{0.5\%} \times SRU_{1.25\%}$; and linear and quadratic regression for SRU 1.25; 2.0 and 3.0%.

in lambs. Subcutaneous fat thickness is an indicator of fat content in the carcass and did not differ among treatments, which is consistent with the nonsignificant differences in carcass traits that were evaluated in the present study. The experimental diets did not influence carcass pH before and after chilling. 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 acceptable, indicating that muscle transformation into meat during chilling occurred as expected, ensuring meat quality (Mancini and Hunt, 2005).

Although the diets varied in their EE content, there were no discernible effects on the meat's chemical composition or quality parameters. Consequently, the potential utilization of SRU in lamb diets seems unrestricted by any adverse impacts on meat quality. The current study observed a linear increase in energy and FA intake with the inclusion of SRU, likely due to better utilization of dietary non-protein nitrogen by cellulolytic and amylolytic ruminal bacteria than in control lambs fed uncoated urea (Russel et al., 1992; Lucena et al., 2024).

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Table 7
Sums of fatty acid groups (mg/100 g of muscle), ratios, and health indices of the *Longissimus lumborum* muscle in lambs fed slow-release urea (SRU) produced from lipid matrix of low-*trans* vegetable fat (LTVF).

Item	Inclusion (% DM total)				SEM[2]	*P*-value[3]		
	$U_{0.5\%}$	$SRU_{1.25\%}$	$SRU_{2.0\%}$	$SRU_{3.0\%}$		$U.0_5 \times SRU_{1.25}$	Linear	Quadratic
Sum (∑) and ratios								
Total FA	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
BI	65.2	64.9	71.7	65.5	6.53	0.979	0.997	0.471
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	0.222	0.003
Total PUFA	131	154	144	148	6.04	0.059	0.620	0.037
n-6 PUFA	110	129	121	125	5.62	0.207	0.632	0.046
n-3 PUFA	11.0	12.2	13.3	13.6	1.33	0.256	0.613	0.247
P/S	0.15	0.20	0.16	0.18	0.02	0.139	0.539	0.547
n–6: *n*–3	11.3	11.6	9.7	9.5	1.19	0.367	0.952	0.194
Indexes								
h/H	1.82	1.81	1.81	1.78	0.05	0.909	0.803	0.814
Atherogenicity	0.54	0.53	0.54	0.54	0.02	0.978	0.785	0.772
Thrombogenicity	89.7	103	102	105	5.31	0.131	0.160	0.257
Enzymatic activity								
SCD17i	39.2	41.3	41.2	37.8	1.90	0.525	0.552	0.199
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 = slow-release urea produced from 60% lipid matrix of low-*trans* vegetable fat (LTVF) and 40% urea; $U_{0.5\%}$ = 5% uncoated urea;
[2]SEM = Standard error of the mean;
[3]*P*-value < 0.05 point to differences between treatments according to orthogonal contrasts: $U_{0.5\%} \times SRU_{1.25\%}$; and linear and quadratic regression for SRU 1.25; 2.0 and 3.0%.

These branched-chain fatty acids (BCFA), including *anteiso*15:0 and *iso*16:0, predominantly originate from the microbial synthesis in the rumen, and previous research (Fievez et al., 2012) suggests that fluctuations in their concentrations in ruminant products reflect changes in ruminal microbiota. Overall, results about BCFA suggest that the LTVF used as the coating material did not harm the growth and reproduction of rumen bacteria. Previously reported negative correlations between rumen concentration of NH_3-N and anteiso17:0 (Cabrita et al., 2003) were not observed in our study. This lack of change in *anteiso*17:0 suggests that SRU diets provided sufficient degradable CP in the rumen, according observed by Lucena et al. (2024).

The impact of dietary lipids on rumen bacteria is contingent on the chain length, unsaturation of their FA, and double bond configuration (Machado et al., 2022). For many bacteria, the incorporation of FA with *trans* double bonds in their membranes serves as a protective mechanism that reduces membrane fluidity and permeability in response to stress stimuli (Bessa et al., 2000; Mauger et al., 2021). Since SRU contains *trans*-FA, the increase in *trans*-MUFA in the meat of lambs fed SRU was expected. However, the linear increase in *trans*-FA did not parallel SRU concentration, indicating the absence of a ruminal microbial stress response.

Beyond the reported health benefits, such as the potential inhibitory action of BCFA on tumor cell synthesis demonstrated by Wongtangtintharn et al. (2004), the increase in BCFA is associated with a distinct "goaty" aroma in cooked meat (Khan et al., 2015), which could be appealing to specific consumers. Rumenic acid (*cis*9, *trans*11–18:2), the major conjugated linoleic acid (CLA) isomer in ruminant tissues, exhibits potent anticarcinogenic effects (Wang et al., 2015). The stability of *cis*9, *trans*11–18:2 concentrations in meat, despite an increase in 18:2*n*-6 intake in $SRU_{1.25\%}$ and $SRU_{2.0\%}$ diets, suggests that SRU might promote a more complete biohydrogenation without altering ruminal biohydrogenation pathways. Incorporating SRU encapsulated in the animals' diet resulted in a 14 mg/100 g increase in total *trans*MUFA (from 26 mg to 40 mg) when SRU was included at 3% ($SRU_{3.0\%}$). However, these increases were not dependent on SRU dose and were quantitatively minor without significantly impacting health indices. This information is relevant for understanding the nutritional and health effects associated with the consumption of meat from ruminants fed SRU.

According to demonstrated by Lucena et al. (2024), the restriction on utilizing the SRU produced here for industrial purposes stems from the melting point of vegetable fat. This can lead to separating phases within the emulsion at elevated temperatures, diminishing the effectiveness of urea protection for gradual release. In addition, it is important to highlight that the energy data (DE and ME) presented in this study is not determined by direct livestock measurements but based on calculation results, which can limit its accuracy.

5. Conclusion

The addition of the urea coated from the low-trans vegetable fat microspheres increased the lamb intake of most fatty acids and energy dietary. However, there was no effect on the carcass traits and meat physicochemical composition. The inclusion of protected urea (SRU) in the lamb diet quadratically increases *trans*-MUFA, *n*-6 PUFA, and total PUFA concentrations in meat, and lambs fed with SRU at level 1.25% in DM total diet presents greater CLA and *trans*-MUFA concentrations compared free urea ($U_{0.5\%}$), which is beneficial from the point of view of the lipid quality of the meat and its relationship with consumer health.

Ethics approval for animal use

All procedures followed the guidelines recommended by the National Ethical Committee for the Control of Animal Experimentation (CONCEA, Brazil) for the use of fistulated animals (Approval Protocol Number 059/2021).

Consent to publication

All authors consent to the publication of this manuscript.

Data availability

The data sets that support the findings of this study are available from the corresponding author upon reasonable request.

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Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the study reported in this paper.

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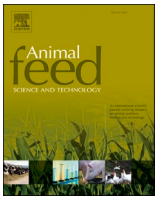

Slow-releasing urea coated with low-*trans* vegetable lipids: Effects on lamb performance, nutrient digestibility, nitrogen balance, and blood parameters

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ABSTRACT

The objective of this study was to produce slow-release urea (SRU) coated with low-trans vegetable fat (LTVF) and compare it to uncoated urea (U) in the diet of lambs. The first experiment evaluated LTVF-coated U produced in three different ratios (U: LTVF), 30:70 (SRU_{30}), 40:60 (SRU_{40}), and 50:50 (SRU_{50}). SRU_{40} had higher ($P < 0.05$) coating yield (85.5 %) and efficiency (98 %) in comparison to SRU_{30} (82.9 and 96 %) and SRU_{50} (83.7 and 96.6 %). SRU_{40} maintained better thermal stability for retention and slower release of urea ($P < 0.05$). A second experiment tested SRU_{40} in 32 intact male Santa Inês lambs (average age: 6 months; average body weight: 17.9 ± 2.01 kg). The lambs were allocated in four treatments: one control diet with 0.5 % U ($U_{0.5\ \%}$) and three levels of SRU_{40} [1.25 % ($SRU_{1.25}$); 2.0 % (SRU_2); 3.0 % (SRU_3); dry matter (DM) basis] in a randomized block design to evaluate performance and ingestive behavior. A third experiment evaluated apparent digestibility, nitrogen (N) metabolism, rumen, and blood

Abbreviations: ADF, acid detergent fiber; ADG, average daily gain; ADL, acid detergent lignin; ADIP, acid detergent insoluble protein; AP, absorbed purines; AST, aspartate aminotransferase; BUN, blood urea nitrogen; BW, body weight; CP, crude protein; DM, dry matter; DMI, dry matter intake; DSC, differential scanning calorimetry; DTG, derived thermogravimetry; EE, ether extract; Feeding efficiency, gain: feed ratio g/g; GGT, gamma glutamyl transferase; LTVF, low-*trans* vegetable fat; NH_3-N, ammonia nitrogen; NDIP, neutral detergent insoluble protein; NPN, non-protein nitrogen; $_a$NDFom, NDF assayed with heat stable amylase and expressed excluding residual ash; NFC, Non-fiber carbohydrates; PD, purine derivates; SFA, saturated fatty acids; SRU, slow-release urea; TG, thermogravimetry; T_i, initial temperature; T_f, final temperature; T_{max}, temperature of highest degradation rate; T_{onset}, Extrapolated initial degradation temperature; Δ_m, mass variation; UFA, unsaturated fatty acids.

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parameters of 24 intact Santa Inês lambs (average age: 6 months; average body weight: 16 ± 2.2 kg) in a randomized block design with six replications. Including SRU linearly increased ether extract (EE), and non-fiber carbohydrates intake ($P < 0.05$) but did not change DM, organic matter, crude protein (CP), and neutral detergent fiber assayed with heat stable amylase and expressed excluding residual ash ($_{a}$NDFom), intake and performance of the lambs ($P > 0.05$). Lambs tended to spend less time ruminating ($P = 0.052$) and more time eating ($P = 0.078$) when SRU was included in the diet. The inclusion of SRU in the diet of lambs tended to linearly increase the apparent total tract digestibility of CP ($P = 0.078$). There was no effect of SRU on N-intake, however, including SRU linearly reduced N-urinary excretion and linearly increased N-retention ($P < 0.05$). Feeding $SRU_{1.25\ \%}$ resulted in higher N-retained and lower N-urinary and N-fecal than feeding $U_{0.5\ \%}$. Blood urea nitrogen (BUN) and rumen pH decreased linearly with the inclusion of SRU, while NH_3-N concentration increased linearly ($P < 0.05$). Lambs fed $SRU_{1.25\ \%}$ presented higher BUN and lower NH_3-N compared to lambs fed $U_{0.5\ \%}$ ($P < 0.05$). N-retained, microbial nitrogen and protein supply linearly increased ($P < 0.05$) due to the inclusion of SRU in the lambs' diet. Lambs fed $U_{0.5\ \%}$ presented the highest ($P < 0.05$) BUN concentrations at all times after feeding. There was a linear decrease of BUN in lambs fed SRU at 0, 4, and 6-h post-feeding ($P < 0.05$). LTVF was efficient in coating urea, especially the formulation with 40 % urea and 60 % vegetable fat (SRU_{40}). It is recommended the inclusion of SRU_{40} in the diet of lambs up to 3 % (total DM basis) as it improves the use of N in the rumen, reducing N-excretion and increasing N-retention, without affecting the performance of the lambs.

1. Introduction

Using non-protein nitrogen (NPN) sources, such as urea, in the diet of ruminants can partially replace true protein sources when used at adequate levels. However, when fed in high quantities, NPN, such as urea, can cause intoxication. Therefore, to reduce the risks and optimize urea utilization, researchers have explored methods to coat it. This enables an increased daily allowance and, consequently, gradual utilization by rumen microorganisms (Geron et al., 2016; Carvalho et al., 2019; de Medeiros et al., 2019; Melo et al., 2021) since they have the ability to transform NPN sources into microbial protein. Since microbial protein represents a major source of amino acids for ruminants, maximizing the efficiency of its production would consequently improve productivity (NRC, 2007.

To produce high-quality slow-release urea (SRU), the coating agent needs to be inert in the rumen, insoluble in water, and hydrophobic. These properties ensure the coating's ability to prevent the rapid release of the coating's core avoiding excess of NH_3-N in the rumen. de Medeiros et al. (2019) and Netto et al. (2021), tested carnauba wax as a coating matrix, while Carvalho et al. (2019) tested beeswax. They demonstrated the feasibility of coating urea o coat urea with a lipid matrix from waxes rich in saturated fatty acids (SFA). This allows urea to be released gradually into the rumen, thereby reducing the risks of toxicity and increasing the availability of NH_3-N for bacteria to produce microbial protein more efficiently. Such advancements have potential to lead to improvements in N balance and animal performance.

Chemical hydrogenation of fatty acids (FA) involves introducing hydrogen to unsaturated fatty acids (UFA), resulting in an increase in saturated fatty acids (SFA), which is done in vegetable oil, for example. Depending on the extent of hydrogenation, it can induce a permanent transition in the state of the oil, changing it from a liquid to a semi-solid or even solid form at room temperature (Wongjaikham, et al., 2022). Hydrogenation has a profound impact on the spreadability of the solidified oil. The production of margarine through partial hydrogenation of vegetable oils, often results in the formation of low-*trans* vegetable fat (LTVF) (Wongjaikham, et al., 2022). LTVF has a higher melting point compared to its original unsaturated form. This characteristic results in a solid or semi-solid texture, similar to saturated fats, which is crucial for the coating process (de Medeiros et al., 2019; Carvalho et al., 2019). Hence, we hypothesized that LTVF will facilitate a controlled release of urea in the rumen due to its physicochemical characteristics. Through this controlled release, we would be able to prevent excessive peaks of NH_3-N in the rumen, potentially enhancing nitrogen balance, improving animal performance, and reducing ammonia release into the environment.

Therefore, the objective of this study was to obtain and investigate the effect of the dietary addition of slow-release urea (SRU) on performance, feed intake, ingestive behavior, apparent digestibility, and N-balance in lambs.

2. Material and methods

2.1. 1st Experiment: production and evaluation of slow-release urea (SRU)

To determine the most effective ratio between U (core) and LTVF (coat), three SRU formulations were produced with different mass/mass ratios between the core and coating agent. The tested ratios (U:LTVF) were: 30:70 (SRU_{30}), 40:60 (SRU_{40}) and 50:50 (SRU_{50}). The coating agent, LTVF (Cukin®, Bunge Alimentos S.A., Brazil), consisted of hydrogenated vegetable oils (mainly soybean oil), antioxidants (terc-butil-hidroquinona and citric acid) and dimethylpolysiloxane defoamer. LTVF had a minimum smoke point of 225 °C (Ribeiro et al., 2009).

Slow-release urea was produced by melt-emulsification (de Medeiros et al., 2019), using soy lecithin as the emulsifying agent at a ratio of 1 % mass of LTVF (Fig. 1a–c).

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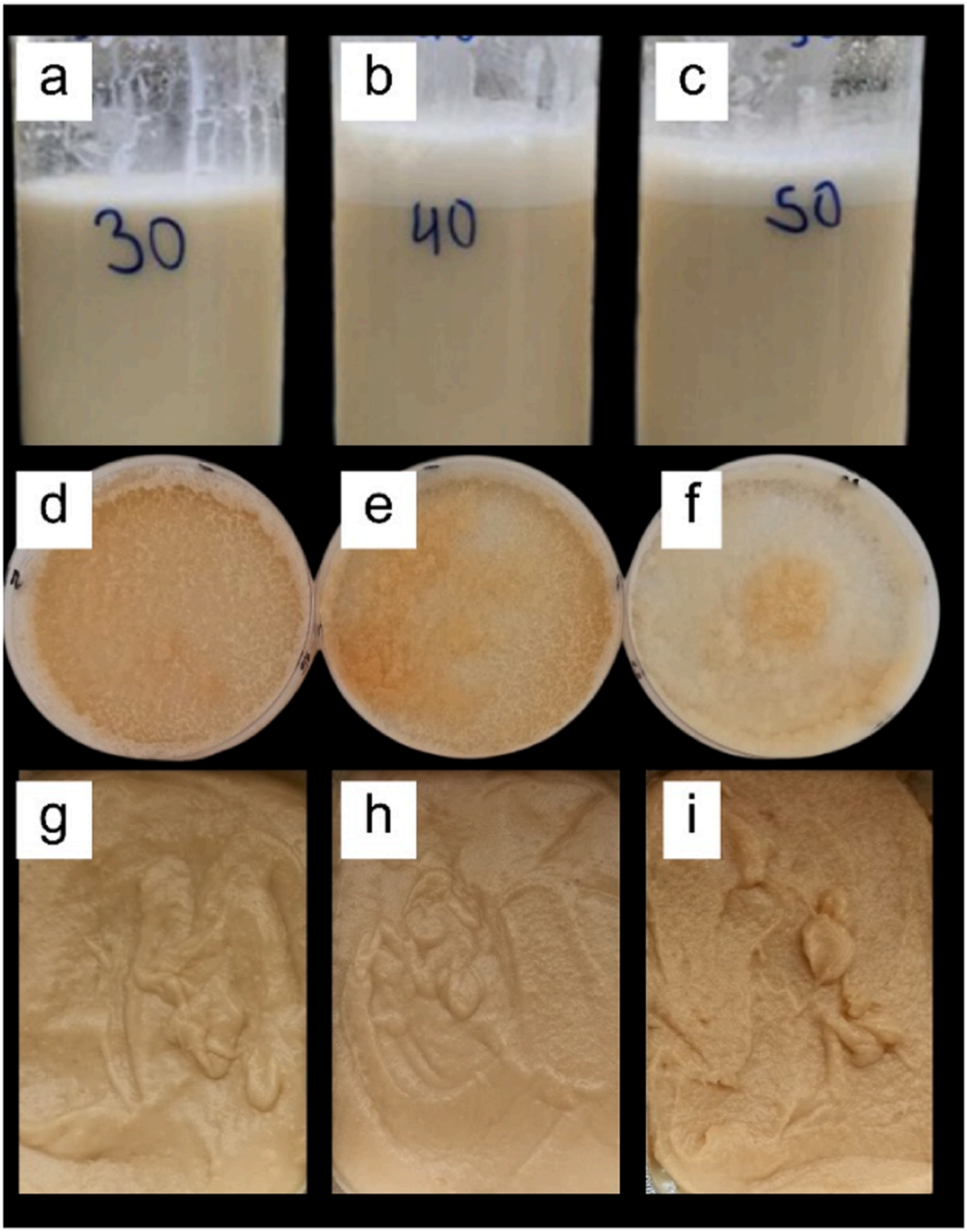

Fig. 1. Slow-release urea (SRU) at ratios U30 %LTVF70 %, U40 %LTVF60 % and U50 %LTVF50 % produced from the melt-emulsification technique using soy lecithin as the emulsifying agent (a = SR_{30}), (b = SR_{40}) and (c = SR_{50}); Emulsion into plastic containers to kept to forced air circulation oven at 55 °C for 24 h before dehydration (d = SR_{30}), (e = SR_{40}) and (f = SR_{50}). SRU stored in a refrigerator at 2 °C for further analysis and use (g = SR_{30}), (h = SR_{40}) and (i = SR_{50}).

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LTVF was weighed on an analytical scale and added to a beaker containing 40 % soy lecithin as a surfactant. The mixture of lecithin and LTVF was kept at 60 °C in a thermostatic bath. Concomitantly, in another beaker, urea was dissolved in distilled water to form a 50 % (w/w) solution with pH of 9.2; and was also kept at 60 °C.

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 homogenizer (T25 digital Ultra-Turrax®, Ika, USA). Finally, the emulsion was transferred to plastic containers and kept in a forced air oven at 55 °C for 24 h (Fig. 1d–f). Once the dried material reached room temperature, it was refrigerated at 2 °C until further analysis (Fig. 1g–i).

The first experiment was arranged as randomized design, consisting of five treatments: three different SRU microparticles ratios (U: LTVF), 30:70 (SRU_{30}), 40:60 (SRU_{40}), and 50:50 (SRU_{50}); uncoated urea (U) and the LTVF used in the SRU preparation.

The coating yield was determined based on the masses of the initially used LTVF, soy lecithin, and 50 % urea solution, as well as the final mass after the drying process. The calculation was performed using the following equation: Yield = $(M_{final}/M_{initial}) \times 100$; 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 coating efficiency represents the capability of LTVF to retain urea. It was calculated by comparing the amount of nitrogen added in the form of urea and the amount that remained after the SRU production processing. The following equation was used: Efficiency = $(U_{retained}/U_{inserted}) \times 100$; where $U_{retained}$ is the urea content retained after SRU production process; $U_{inserted}$ is the urea content inserted into the system.

Derivative thermogravimetric (DTG) curves were obtained using a thermogravimetric analyzer (model TGA-50 Shimadzu Corporation®, Kyoto, Japan), under an inert atmosphere (Argon), at a flow rate of 50 mL/min. The heating rate was set at 10 °C/min, covering a temperature range of 30–600 °C. An alumina crucible containing an average of 6.0 mg of sample (DM basis), as a function of temperature and time simultaneously (de Medeiros et al., 2019; Carvalho et al., 2019; Melo et al., 2021). The DTG curves and data analysis were performed using OriginPro 8 software, considering T_{onset} as the initial degradation temperature to obtain the variation of thermal degradation of materials (SRU, core and coat).

Differential scanning calorimetry (DSC) curves were obtained in a differential scanning calorimeter (model DSC-60, Shimadzu Corporation®, Kyoto, Japan) under an inert atmosphere (Argon), in a flow of 50 mL/min. The heating rate of 10 °C/min, covering a temperature range of 30–300 °C. A platinum crucible containing around 2.5 mg of the sample was used. The plotting of the DSC curves and data analysis were performed using OriginPro 8 software, considering the peak temperature of the events (de Medeiros et al., 2019; Carvalho et al., 2019; Melo et al., 2021).

2.2. 2nd Experiment: performance and ingestive behavior

This study was conducted at the Federal University of Campina Grande (UFCG), Paraiba state, Brazil, strictly following the recommendations of the Guide for the Care and use of Animals in Research and Teaching. The research protocol was approved by the Committee on the Ethics of Animal Experiments of UFCG, Brazil, under Protocol Number 059/2021.

Thirty-two intact Santa Inês lambs, with an average of 6-months of age and initial weight of 17.9 ± 2.01 kg, were included in a randomized block design trial. The study comprised of four treatments and eight lambs assigned to each treatment. The initial weight was used as criterion to form two blocks. The treatments included the control ($U_{0.5\ \%}$), consisting of the inclusion of 0.5 % urea in the total DM. The other treatments involved the addition of SRU coated by LTVF at proportions of 1.25 % ($SRU_{1.25\ \%}$); 2.0 % ($SRU_{2\ \%}$) and 3.0 % ($SRU_{3\ \%}$) of the total DM of the diet. These proportions corresponded, respectively, to 0.5 % (similar to the control treatment); 0.8 and 1.2 % of urea in the diet.

The experiment lasted 75-d, including 60-d performance evaluation phase. Prior to the beginning of the experiment, lambs were weighed, vaccinated against clostridium disease (Biovet Resguard Multi®, São Paulo, Brazil), orally dewormed with 5 % Levamisole hydrochloride (Ripercol® L, São Paulo, Brazil) and orally supplemented with a vitamin mix (A, D and E). The lambs were then distributed in individual wooden and suspended pens (1.75 m × 2.0 m) equipped with feeding and drinking troughs.

The experimental diets were formulated to meet the requirements of growing intact male sheep for an average gain of 200 g/d as recommended by NRC (2007). The roughage source (300 g/kg DM) was Tifton-85 (*Cynodon* sp) hay, while the concentrate (700 g/kg

Table 1
Chemical composition of ingredients used in experimental diets.

Item	Ground corn silage[a]	Urea	SRU[b]	Soybean meal	Ground corn	Tifton–85 hay
Dry matter (g/kg as fed)	670	980	981	916	899	871
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
$_{a}$NDFom[c]	127	-	-	157	115	728
Non-fiber carbohydrates	718	-	-	345	701	91.3
Cellulose	105	-	-	89.2	75.7	265
Hemicellulose	19.4	-	-	59.6	31.0	411
Acid detergent lignin	3.22	-	-	8.23	8.32	52.5

[a] Hydrated with cactus pear mucilage.
[b] SRU = slow-release urea produced from 60 % of lipid matrix of low-*trans* vegetable fat (LTVF) and 40 % urea (U).
[c] NDF assayed with a heat stable amylase and expressed exclusive of residual ash.

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DM) was composed of ground corn, ground corn silage hydrated with cactus pear mucilage, soybean meal, urea, coated urea with LTVF and mineral mix (Table 1). Ground corn silage was produced using cactus pear (*Opuntia stricta*) as the humectant in a corn grain to cactus pear mucilage ratio of 75:25. Following mixing, the material was compacted in plastic barrels, sealed, and ensiled for 90 d (Tables 2 and 3).

Diet was offered as total mixed ration (TMR) into two equal parts provided to the lambs at 7h00 and 17h00. Feed and water were available *ad libitum*. Urea and SRU_{40} were added to the diet based on total DM and homogenized in a Y-type mixing wagon (Coppi®, Santa Catarina, Brazil). To facilitate adaptation, urea was gradually introduced to the lambs for 30 d. The daily amount of feed offered was adjusted based on previous day's intake to allow leftovers of approximately 10 %. Samples of diets and leftovers were collected daily, pooled into a weekly composite for each animal, and frozen at –20 °C for further analysis. The lambs were individually housed in stalls equipped with drinking and feeding troughs.

Dry matter intake (DMI) was calculated based on the amounts of feed offered and the recorded leftovers during the experimental period. To assess the selectivity of the diet by the lambs regarding urea consumption, the chemical composition of the effectively consumed diet was determined by dividing the intake of each nutrient by the DMI and multiplying by 100 (Mazza et al., 2020).

At the start and conclusion of the experiment, individual lamb weights were recorded to calculate the total weight gain (TWG, kg) and average daily gain (ADG, g/d). Following a 12-h solid feed fasting at the end of the experimental period, lambs were weighed again to determine TWG. Feeding efficiency was determined as the ratio between ADG and DMI (ADG/DMI).

On days 21 and 42, individual observations of the lambs were conducted over 24 h at 5-min intervals to assess ingestive behavior, following the method outlined by Martin and Bateson (1993). Two trained observers recorded data on the behavioral activities of each animal, minimizing interference with the lambs' behavior. Observers alternated shifts every three hours, and nighttime observations were conducted under artificial lighting. To estimate the average number of boli chewed per d, the number of chews per bolus and chewing time for each bolus, evaluations were performed in three distinct periods from 10h00 to 12h00, 14h00 to 16h00 and 18h00 to 20h00 (Beauchemin, 2018). Eating (g DM/h) and rumination efficiency (g $_{a}$NDFom/h) were calculated according to Bürger et al. (2000). Total chewing time (TCT, h/d) was calculated as the sum of eating and ruminating time.

2.3. 3rd Experiment: apparent digestibility, nitrogen balance, rumen and blood parameters

Twenty-four lambs, with an average age of 6 mo and an average weight of 16 ± 2.2 kg, were individually housed in metabolic crates equipped with drinking and feeding troughs, as well as a compartment for a total collection of feces and urine. The experiment lasted 28-d, with a 21-d for adaptation of lambs to the environment, management, and diets, followed by a 7-d for collection of leftovers, feces, and urine. The diets administered during this third experiment were identical to those used in the lamb growth

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

Item	U (%DM)	SRU_{40}[a] (%DM)		
	0.5	1.25	2.0	3.0
Ingredient proportion (g/kg DM)				
Tifton–85 hay	300	300	300	300
Ground corn	505	498	518	539
Ground corn silage hydrated[b]	20	20	20	20
Soybean meal	140	139.5	112	81
Urea (U)	5.0	-	-	-
Slow-release urea (SRU_{40})[a]	-	12.5	20	30
Mineral mixture[c]	30	30	30	30
Chemical composition of the 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
$_{a}$NDFom[d]	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[e]	312	310	312	314
Acid detergent insoluble protein[e]	194	192	196	199

[a] SRU = slow-release urea produced from 60 % of lipid matrix of low-*trans* vegetable fat (LTVF) and 40 % urea (U).
[b] Hydrated with cactus pear mucilage.
[c] 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, and maximum 870 mg of fluoride.
[d] $_{a}$NDF assayed with a heat stable amylase and expressed exclusive of residual ash.
[e] (g/kg CP).

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Table 3
Fatty acids profile (g/100 g total FA) of low-*trans* vegetable fat (LTVF) and different formulations of slow-release urea (SRU) produced from the lipid matrix of LTVF.

FA composition	Low-*trans* vegetable fat	Slow-release urea (SRU)[a]		
		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
C18:2*n*-6	48.2	26.0	26.8	27.7
C20:0	0.34	0.35	0.34	0.31
C18:3*n*-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

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

performance study (second experiment).

During the seven days of collection, the total urine produced by the lambs in a 24-h period was collected in a plastic reservoir containing 50 mL of a 20 % sulfuric acid solution. After collection, an aliquot of 10 % of the total urine value was subsampled and kept at pH below three to prevent degradation of metabolites present in the urine. These samples were stored in plastic containers at –20°C and later used for the determination of total nitrogen concentrations. Additionally, samples the feed used to compose the experimental diets, as well as leftovers and feces, were dried in a forced-air circulation oven at 55°C for 72 h.

Urinary allantoin concentration was determined by the Rimini-Schryver reaction using the colorimetric method described by Young and Conway (1942). The determination of uric acid was carried out using a commercial kit (Labtest®, São Paulo, Brazil) using the Trinder reaction (Fossati et al., 1980). Excreted purine derivatives (PD) was calculated as the sum of daily urinary excretion of allantoin and uric acid, without considering excretion of xanthine and hypoxanthine. This is due to the fact that allantoin and uric acid are highly correlated with nucleic acid concentration in the rumen (Topps and Elliott, 1965; Santos et al., 2022), and the values of xanthine and hypoxanthine in small ruminants contribute to no more than 1 % of the total PD. Absorbed PD were calculated according to Chen et al. (1990). Microbial nitrogen supply (MNS) was calculated according to Chen et al. (1992). Microbial protein supply (MPS) was calculated as MNS × 6.25.

Nitrogen retention was calculated as follows: N-retained (g/d) = N-intake (g/d) – N-fecal excretion (g/d) – N-urinary excretion (g/d).

A total of 150 mL of rumen fluid was collected during the apparent digestibility assay. Collection was performed using a 1.5 m long flexible tube with an internal diameter of 1.27 cm and a wall thickness of 0.3 cm. The probe had a metal nozzle with a closed end and holes on the sides. To prevent cross-contamination between animals, the probe was lubricated with petroleum jelly and rinsed with distilled water between collections. Rumen fluid was collected four h after the morning feeding. The first aliquot was discarded to avoid contamination with saliva. Immediately after collection, the fluid was filtered through double layers of gauze, before the pH was measured (pH meter model 205, Testo®, Brazil). Aliquots of 50 mL of the rumen fluid were stored in plastic tubes acidified with 1.0 mL of 50 % (vol/vol) sulfuric acid (H_2SO_4) solution and frozen at –20°C for subsequent determination of estimated NH_3-N contents through the Kjeldhal method (AOAC, 2015).

Blood was collected from all lambs on the last day of the experimental period through jugular vein puncture using disposable needles (21 mm × 1 mm) (Vacuette®, Greiner Bio-One, Austria) immediately before feeding (0 h), and 2, 4 and 6 h after feeding. To facilitate collection and ensure animal welfare, the lambs were trichotomized in the region of the external jugular vein and a n° 16 catheter (Medical supply®, São Paulo, Brazil) was inserted in each lamb. The blood samples were temporarily kept at room temperature until the clot was formed and then centrifuged at 2500 × g for 5 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. Serum metabolites were measured using commercial kit tests (Labtest®, São Paulo, Brazil) for total protein, albumin, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), cholesterol, triglycerides, calcium (Ca), phosphorus (P) and magnesium (Mg). The measures were obtained using a Cobas C111 automatic biochemical analyzer (Roche, Germany) in enzymatic or colorimetric kinetic assays.

2.4. Chemical analyses

Samples of the feed used to compose the experimental diets, leftovers and feces were dried in a forced air circulation oven at 55 °C for 72 h. Subsequently, all feeds were processed in a Wiley knife mill, using a sieve of 1.0 mm, and analyzed for DM content (method

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967.03), mineral matter (MM - method 942.05), crude protein (CP - method 981.10), and ether extract (EE - method 920.29), according to AOAC (2015)

To determine the contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF), the methodology of Van Soest et al. (1991) was used with modifications for nonwoven filter bags (Senger et al., 2008), with the inclusion of thermostable amylase (Sigma A3306; Sigma–Aldrich, Steinheim, Germany) and expressed exclusive of residual ash ($_a$NDFom). The hemicellulose content was derived by subtracting ADF from the NDF, while the cellulose content was determined by subtracting the lignin from the ADF. These calculations were performed through sequential analyses on the same sample.

The residue from the neutral detergent boiling was incinerated in a muffle furnace at 600 °C for 4 h, and the correction for CP was carried out by discounting the neutral detergent insoluble protein (NDIP) content. Lignin was determined by treating the ADF residue with 72 % sulfuric acid. The content of NDIP and acid detergent insoluble protein (ADIP) were obtained according to Licitra et al. (1996).

Non-fiber carbohydrates (NFC) were estimated by the equation suggested by Hall (2003): NFC (g DM/kg) = 1000 – [(CP – CP_u + U) + $_a$NDFom + EE + ash], where CP is the crude protein content, CP_u is the crude protein derived from urea, U is the urea content, NDF is the neutral detergent fiber content adjusted for ash and nitrogen compounds, and EE is the ether extract content; the value of NDF corrected for ash and protein was considered in the calculation (Mertens, 1997).

2.5. Statistical analyses

The first experiment, designed to evaluate the urea coating system, was analyzed as a completely randomized design with five treatments: SRU_{30}, SRU_{40}, SRU_{50}, U and 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 a 5 % significance level) through the PROC MIXED procedure of SAS (version 9.1; SAS Institute, Cary, NC, 2003).

For the second and third experiments, data were analyzed in randomized block design, with four treatments (0.5 inclusion of free urea as control and SRU coated by LTVF at the proportions of 1.25; 2.0 and 3.0 % of DM) with eight lambs and six replications (lambs in metabolic cages), respectively. Each animal was considered an experimental unit. The blocks were formed based on the body weight of the lambs at the beginning of the experiment. Data analysis was carried out using the SAS® procedures (version 9.1; SAS Institute, Cary, NC, 2003).

The homoscedasticity of the data was tested using Bartlett's method, and the normality of errors using the Shapiro-Wilk test. Records were then subjected to analysis of variance, considering fixed effects of treatment, and random effects of block and experimental error through the PROC MIXED procedure of SAS (version 9.1; SAS Institute, Cary, NC, 2003).

The following statistical model was used:

$$Y_{ijk} = \mu + \tau_i + \beta_j + \varepsilon_{ijk},$$

where Y_{ijk} = dependent variable, μ = general mean, τ_i = fixed effect of i_{th} level of replacement (i = 1–4), β_j = random effect of j_{th} block (j = 1–4) and ε_{ijk}= random error associated to each observation.

The obtained data were subjected to analysis of variance using the PROC MIXED procedure of SAS (version 9.1; SAS Institute, Cary, NC, 2003). Means were compared using orthogonal contrasts, estimated through PROC IML procedure of SAS (version 9.1; SAS Institute, Cary, NC, 2003). These defined contrasts were used to separately compare the control treatment ($U_{0.5\ \%}$) to $SRU_{1.25}$, as they had the same theoretical amount of urea but with different coating (uncoated or coated urea). This comparison aimed to assess the effect of vegetable fat on the efficiency of urea coating. Additionally, linear and quadratic contrasts were examined within the three levels of SRU_{40} inclusion (1.25 %, 2 %, and 3 %). Differences were considered significant when $P < 0.05$.

3. Results

Three SRUs demonstrated coating yields above 80 %, with SRU40 exhibiting the highest coating efficiency at 85.5 %. It retained the highest percentage of U when compared to SRU30 and SRU50. This indicates that the formulation with 40 % urea and 60 % LTVF was the most efficient coating, despite its higher moisture content and intermediate crude protein concentration (115 %) compared to the

Table 4
Yield and efficiency, crude protein and moisture of slow-release urea (SRU) produced from lipid matrix of low-trans vegetable fat (LTVF) at different proportions.

	Slow-Release Urea (SRU)[a]			SEM[2]	*P*-value[2]
Item (g/kg DM)	SRU_{30}	SRU_{40}	SRU_{50}		
Yield	829c	855 a	837 b	0.20	0.023
Efficiency	960 b	980 a	966 b	4.91	0.002
Crude protein	802c	1154 b	1342 a	27.4	0.001
Moisture	56.8 b	62.1 a	53.1 b	7.22	0.039

Means followed by the same letters do not differ according to Tukey's test; significant at $P \leq 0.05$.

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

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other SRUs (Table 4).

In the DTG (Fig. 2a) and DSC (Fig. 2b) thermal analyses of the coating (LTVF) and core materials (urea) used in the processing, it was observed that urea showed two main thermal degradation events with T_{onset} of 187 °C in 16.95 min. In contrast, LTVF showed a single degradation event with T_{onset} of 381 °C in 36.06 min. Upon 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. The T_{onset} temperatures for these formulations were 165 °C in 14.18 min for SRU_{30}, 168 °C in 14.43 min for SRU_{40}, and 160 °C in 13.86 min for SRU_{50}.

Among the formulations, SRU_{40} was the only one that exhibited an event before thermal degradation, attributed to a higher moisture content (6.21 %) compared to the other SRU. LTVF proved to be an efficient coating agent for urea, with SRU_{40} showing the highest initial temperatures and degradation times. In contrast, SRU_{50}, with higher urea inclusion, showed lower initial temperatures and degradation times. This effect is further emphasized by the T_{max} of 202 °C in 18.01 min, 208 °C in 18.75 min, and 192 °C in 17.25 min for SRU_{30}, SRU_{40} and SRU_{50}, respectively. These values imply that higher temperature and longer times are required for the highest degradation rate in SRU_{40} and lower for SRU_{50}.

In terms of thermal behavior in DSC, urea exhibited one endothermic event corresponding to its melting point at 135 °C. Additionally, two overlapping endothermic events with peaks at 206 °C and 226 °C were observed, associated with its thermal degradation. In contrast, LTVF showed no thermal events within the evaluated temperature range of 30–300 °C. For the coated 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. The events including the initiation of thermal degradation of urea in the coated 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 SRU changed the FA profile of the LTVF used as coating for urea (Table 4). This change led to a reduction, mainly in C18:2*n*-6 and C18:3*n*-3 and increase in C18:1-*trans*, C18:1-*cis* and C18:2. The different concentrations of LTVF in the three formulations did not change the FA profile.

Based on the results of the first experiment, particularly with the thermal analyses revealing a more gradual degradation of the core, the coated SRU_{40} formulation was selected for *in vivo* additional tests.

The inclusion of SRU did not alter the intakes of DM, OM, CP and $_{a}$NDFom, as well as the composition of the DM and CP effectively consumed by the lambs (Table 5). However, there was a linear increase in the daily intake of EE ($P = 0.019$), and NFC ($P = 0.029$), with a linear decrease in the composition of the $_{a}$NDFom effectively consumed ($P = 0.039$) due to the inclusion of SRU in the lambs' diet.

$SRU_{1.25\ \%}$ promoted a higher EE intake if compared to $U_{0.5\ \%}$ ($P < 0.05$). The inclusion of SRU in the diet of lambs tended to linearly increase the total tract apparent digestibility of CP ($P = 0.078$) due to SRU inclusion, with no difference between the CP total apparent digestibility of $U_{0.5\ \%}$ and $SRU_{1.25\ \%}$. The apparent digestibility of the other nutrients evaluated did not change with the addition of the SRU to the diet ($P > 0.05$).

There was no difference in performance parameters with the inclusion of SRU in the diet ($P > 0.05$). The lambs had an average final weight of 30.4 kg with final weight gain of 12.4 kg and an ADG of 207 g.

The time spent idling ($P > 0.05$) was not affected by SRU (Table 6). However, there was a trend for linear reduction ($P = 0.052$) in the time spent ruminating. Additionally, for the time spent eating, it was observed a trend for linear increase ($P = 0.078$) with the inclusion of SRU in the lambs' diet. The efficiency rate of eating and rumination of DM and $_{a}$NDFom, the number of boli ruminated per d, and the amount of DM ruminated per bolus were not affected by the inclusion of SRU in the diet ($P > 0.05$). There was no difference between $U_{0.5\ \%}$ and $SRU_{1.25\ \%}$ for the ingestive behavior variables ($P > 0.05$).

The mean daily N-intake was 19.7 g (Table 7), and there was no effect of SRU inclusion in the diet of lambs ($P = 0.92$). However, the inclusion of SRU linearly reduced N-urinary excretion ($P = 0.038$) and N-urinary excretion as %N-intake ($P = 0.001$), while linearly increasing N-retained as g/d and %N-intake ($P < 0.05$). When comparing $U_{0.5} \times SRU_{1.25\ \%}$, the urea coated by LTVF provided greater efficiency of N utilization, causing less excretion of urinary-N ($P = 0.047$) and fecal-N ($P = 0.008$) and higher nitrogen retention ($P = 0.028$). There was a linear increase ($P = 0.014$) in microbial nitrogen and protein supply with the inclusion of SRU in the diet of lambs.

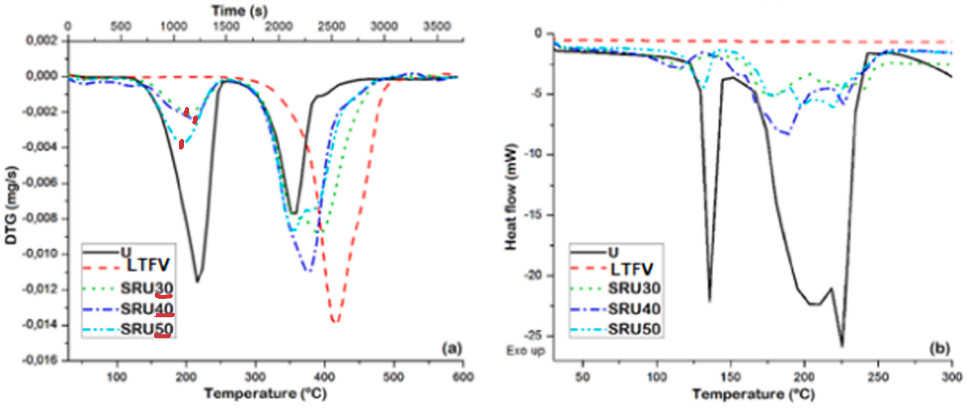

Fig. 2. (a) Thermogravimetric curves (DTG curves) and (b) Differential scanning calorimetry curves (DSC curves) for free urea (U), low-*trans* vegetable fat (LTVF) and urea systems protected by vegetable fat (SRU_{30}, SRU_{40}, and SRU_{50}).

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Table 5
Intake, composition of the diet effectively consumed, apparent digestibility, and growth performance of lambs fed slow-release urea (SRU) produced from 60 % lipid matrix of low-*trans* vegetable fat (LTVF) and 40 % urea (U).

Variable	U	SRU_{40}[a] (% total DM)			SEM[b]	*P*-value[c]		
	0.5	1.25	2	3		$U_{0.5} \times SRU_{1.25}$	Linear	Quadratic
Intake (g/d)								
Dry matter	843	813	856	871	74.6	0.89	0.58	0.97
Organic matter	783	755	797	811	68.7	0.88	0.57	0.96
Crude protein	120	119	125	129	12.1	0.79	0.55	0.78
$_{a}$NDFom[d]	203	213	195	197	17.3	0.94	0.76	0.50
Ether extract	39.4	43.7	53.1	61.8	2.84	0.005	0.019	0.18
Non-fiber carbohydrates	406	404	431	462	44.9	0.64	0.029	0.55
Composition of the diet effectively consumed (g/kg DM)								
Organic matter	927	928	930	932	13.8	0.94	0.71	0.37
Crude protein	146	142	144	148	15.0	0.28	0.54	0.15
$_{a}$NDFom[d]	255	254	233	222	4.82	0.43	0.039	0.12
Ether extract	46.7	53.5	60.9	71.2	1.06	< 0.001	< 0.001	0.55
Apparent digestibility coefficient (g/100 g ingested)								
Dry matter	73.3	73.2	74.8	73.4	1.73	0.59	0.91	0.99
Organic matter	75.1	74.9	76.3	75.3	1.67	0.68	0.85	0.99
Crude protein	63.2	66.9	65.5	67.5	1.78	0.45	0.92	0.078
$_{a}$Neutral detergent fiber[d]	52.9	50.4	48.3	45.4	3.96	0.61	0.43	0.28
Ether extract	88.8	90.0	91.3	90.8	4.54	0.69	0.61	0.13
Non-fiber carbohydrates	87.5	87.5	90.9	89.5	9.57	0.35	0.28	0.43
Growth performance								
Initial body weight (kg)	18.2	17.5	18.2	18.0	-	-	-	-
Final body weight (kg)	31.0	29.9	30.5	30.0	1.42	0.81	0.93	0.56
Total weight gain (kg)	12.8	12.4	12.3	12.0	0.98	0.81	0.79	0.63
Average daily gain (g)	213	207	206	200	1.63	0.81	0.80	0.64
Feeding efficiency (kg/kg)	0.25	0.25	0.24	0.23	0.18	0.96	0.93	0.98

[a] SRU_{40} = slow-release urea produced from 60 % lipid matrix of low-*trans* vegetable fat (LTVF) and 40 % urea (U).
[b] SEM = Standard error of the mean.
[c] *P*-value < 0.05 point to differences between treatments according to orthogonal contrasts: $U_{0.5\ \%} \times SRU_{1.25\ \%}$; and linear and quadratic regression.
[d] NDF assayed with a heat stable amylase and expressed exclusive of residual ash.

Table 6
Ingestive behavior of lambs fed diets containing slow-release urea (SRU) produced from 60 % lipid matrix of low-*trans* vegetable fat (LTVF) and 40 % urea (U).

Variables	U	SRU_{40}[a] (% total DM)			SEM[b]	*P*-value[c]		
	0.5	1.25	2	3		$U_{0.5} \times SRU_{1.25}$	Linear	Quadratic
Spent time (min/d)								
Eating	383	335	408	403	35.3	0.24	0.078	0.87
Ruminating	215	190	173	168	14.9	0.11	0.052	0.11
Idling	833	888	838	875	33.2	0.19	0.40	0.34
Efficiency rate (g/h)								
Eating DM	296	305	356	396	38.8	0.82	0.36	0.26
Eating $_{a}$NDFom	73.0	75.0	81.0	90.0	15.4	0.83	0.47	0.44
Ruminating DM	170	182	148	143	15.3	0.76	0.27	0.67
Ruminating $_{a}$NDFom	40.4	43.0	34.5	32.1	8.76	0.7	0.17	0.53
Chewing								
Amount (g DM/bolus)	544	458	517	525	38.9	0.16	0.29	0.32
Ruminated boli (n°/d)	1.88	2.21	1.87	1.82	0.21	0.48	0.34	0.84

[4]NDF assayed with a heat stable amylase and expressed exclusive of residual ash.
[a] SRU_{40} = slow-release urea produced from 60 % lipid matrix of low-*trans* vegetable fat (LTVF) and 40 % urea (U).
[b] SEM = Standard error of the mean.
[c] *P*-value < 0.05 point to differences between treatments according to orthogonal contrasts: $U_{0.5\ \%} \times SRU_{1.25\ \%}$; and linear and quadratic regression.

Serum urea concentration and rumen pH decreased linearly (P < 0.001) with SRU inclusion. In contrast, the NH_3-N concentration increased linearly (P = 0.026). The inclusion of SRU also 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) when compared to control ($U_{0.5\ \%}$), and $SRU_{1.25}$ tended (P = 0.059) to present lower serum magnesium concentration when compared to $U_{0.5\ \%}$ (Table 8). Serum concentrations of albumin, GGT, creatinine, cholesterol, triglycerides, calcium, and phosphorus were not affected by SRU inclusion.

BUN (Fig. 3.a) linearly decreased according to the form of offer. The highest BUN concentrations were observed for $U_{0.5\ \%}$ (control) at 0, 4 and 6 h after feeding (P < 0.05). All SRU diets promoted BUN peak at 2 h after feeding, showing similar concentration to $U_{0.5\ \%}$

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Table 7
Nitrogen metabolism, rumen parameters and microbial protein synthesis in lambs fed slow-release urea (SRU) produced from 60 % lipid matrix of low-*trans* vegetable fat (LTVF) and 40 % urea (U).

Variables (g/d)	U	SRU_{40}[a] (% total DM)			SEM[b]	*P*-value[c]		
	0.5	1.25	2.0	3.0		$U_{0.5}$×$SRU_{1.25}$	Linear	Quadratic
N-intake	19.3	19.0	20.0	20.7	0.92	0.79	0.55	0.78
N-fecal	7.11	6.4	6.96	6.77	0.86	0.008	0.73	0.64
N-urinary	5.52	4.85	4.63	3.76	0.83	0.047	0.038	0.44
N-retained	6.67	7.75	8.41	10.2	0.75	0.028	0.019	0.31
N-Exc. %N-intake	65.4	59.2	58.0	50.9	4.85	0.98	0.001	0.49
N-Ret. % N-intake	34.6	40.8	42.0	49.1	4.83	0.98	0.021	0.53
NH_3-N (mg/dL)	26.6	25.2	26.5	35.5	3.04	0.76	0.03	0.98
Rumen pH	6.12	5.92	5.43	5.48	0.11	0.70	< 0.001	0.08
Microbial nitrogen supply	4.67	4.74	4.96	5.31	0.42	0.88	0.014	0.52
Microbial protein supply	29.2	29.6	31.1	33.2	2.65	0.89	0.014	0.53

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

Table 8
Blood metabolites of lambs fed slow-release urea (SRU_{40}) produced from 60 % lipid matrix of low-*trans* vegetable fat (LTVF) and 40 % urea (U) as core.

Blood variables	U	SRU_{40}[a] (% total DM)			SEM[b]	*P*-value[c]		
	0.5	1.25	2	3		$U_{0.5}$×$SRU_{1.25}$	Linear	Quadratic
Total protein (g/dL)	5.41	5.21	5.29	5.58	0.15	0.183	0.096	0.969
Albumin (g/dL)	3.39	3.44	3.53	3.47	0.11	0.683	0.887	0.663
AST (U/L)	72.9	70.2	71.4	76.9	2.70	0.159	0.092	0.727
GGT (U/L)	57.0	52.3	54.5	58.8	3.30	0.365	0.166	0.802
Creatinine (mg/dL)	0.58	0.51	0.55	0.51	0.04	0.542	0.923	0.246
BUN (mg/dL)	37.1	32.0	29.1	24.5	1.61	0.045	0.009	0.324
Cholesterol (mg/dL)	31.6	36.7	35.8	34.3	2.62	0.673	0.453	0.263
Triglycerides (mg/dL)	25.9	25.0	32.0	27.6	2.76	0.265	0.529	0.870
Calcium (Ca, mg/dL)	6.06	6.67	5.99	6.40	0.45	0.520	0.629	0.409
Phosphorus (P, mg/dL)	7.19	7.05	7.39	6.93	0.21	0.134	0.736	0.438
Magnesium (Mg, mg/dL)	1.07	0.77	1.30	0.88	0.15	0.059	0.530	0.205

AST = aspartate Aminotransferase, GGT = Gamma Glutamyl Transferase, BUN = Blood Urea Nitrogen.
[a] SRU_{40} = slow-release urea produced from 60 % lipid matrix of low-*trans* vegetable fat (LTVF) and 40 % urea (U).
[b] SEM = Standard error of the mean.
[c] *P*-value < 0.05 point to differences between treatments according to orthogonal contrasts: $U_{0.5\ \%}$ × $SRU_{1.25\ \%}$; and linear and quadratic regression.

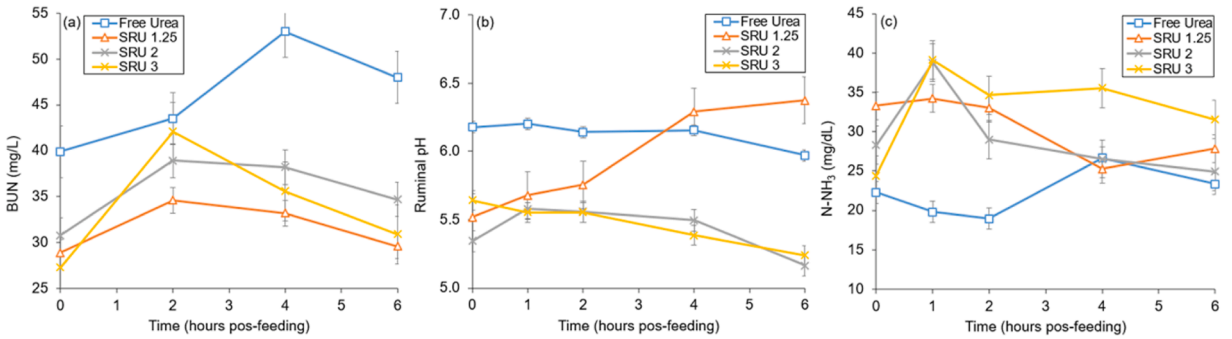

Fig. 3. Means over hours post-feeding of blood urea nitrogen (BUN) (a) rumen pH (b) and ammonia nitrogen (NH_3-N) (c) in lambs (n = 32) fed slow-release urea (SRU_{40}) core protected by the low-*trans* vegetable fat matrix (40 % of U: 60 % of LTVF ratio) and added in 1.25 % ($SRU_{1.25}$), 2 % (SRU_2) and 3 %(SRU_3) in comparison to free urea (U).

(P = 0.117). The diets with SRU showed the lowest values and variations of BUN concentration over time compared to the diet with $U_{0.5\ \%}$.

Regarding rumen parameters, the inclusion of SRU altered the pH (Fig. 3.b) and NH_3-N concentration (Fig. 3.c), particularly 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),

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although 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 a linear increase was observed at 1, 2, 4 and 6 h after the first feeding ($P < 0.05$).

4. Discussion

The high coating yields (>82 %) and efficiency (>95 %) observed in SRU production, particularly for SRU_{40}, underscore the efficacy of the melt-emulsification technique in coating urea using LTVF as the coating material. These results indicate that melt-emulsification can efficiently coat urea during processing, minimizing potential losses. Furthermore, LTVF proved to be stable as a urea coating, showing no endothermic events when evaluated alone, which confirms its suitability for the coating process.

However, when comparing LTVF to other coatings (i.e.: beeswax and carnauba wax) previously published, the waxes showed higher coating yield (de Medeiros et al., 2019; Carvalho et al., 2019). Carvalho et al. (2019) tested a 50:50 ratio of beeswax coating on urea and achieved an efficiency of 92.5 %. Netto et al. (2021) tested urea coated with carnauba wax at a 50:50 and 25:75 ratio and observed a decrease in the proportion of urea incorporated into the material with coating yields ranging from 92.1 % to 87.5 %. Netto et al. (2021) attributed this reduction to the higher viscosity of the wax, potentially causing material loss through adherence to the containers' s walls used during the preparation process. This would not happen with LTVF, as it is less viscous.

During the production of SRU, it was necessary to add water to solubilize the urea and enable emulsification with LTVF, utilizing lecithin as emulsifier. However, during the dehydration process, water is removed from the system, potentially leading to nitrogen losses through evaporation, with some of the nitrogen present in the urea being carried away. These losses account for coating efficiencies being less than 100 % and for the presence of residual moisture in the final material. Although complete water removal was not achieved in any of the formulations, due to the hygroscopic nature of the urea and the barrier formed by the LTVF, preventing the total evaporation of water, the moisture content present in the final material was not harmful, as confirmed by the thermal analyses.

The thermal degradation events data indicate a combination of the degradation patterns between urea and LTVF. In turn, this combination suggests that all formulations used to coat urea with vegetable fat were efficient, as there was an increase in the temperature of the main degradation stage associated with urea degradation. Notably, SRU_{40} was more efficient in increasing this temperature when compared to SRU_{30} and SRU_{50}.

DSC results suggested that SRU_{40} exhibits better thermal stability because the thermal degradation events started to happen at a higher temperature and only at two time points, unlike SRU_{30} and $SRU_{50,}$ which presented three events starting at lower temperatures. Moreover, the higher stability corroborates with observations from TG and DTG analysis. The higher thermal stability for SRU_{40} indicates a limit on the amount of core (urea) that LTVF can coat, with stability loss when urea increased in the SRU_{50} formulation. This decline in the stability of SRU_{50} may also have occurred due to two main factors: the higher water content 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 indicates a potential decrease in the emulsion strength. According to Jiang et al. (2018), an interfacial membrane of the lower strength emulsion can easily be subjected to the perforation of fat crystals, as well as the distortion and rupture of some emulsion droplets. Consequently, this can lead to phase separation between the shell and core materials, compromising the SRU coating.

SRU produced with LTVF needs to be stored under refrigeration before being mixed with the feed to ensure the stability of emulsion. When stored correctly, the material has a shelf life of 30 days; after this period, it is no longer possible to ensure the stability of the emulsion. Another factor that may interfere with this stability is that once manufactured, emulsions naturally undergo complete separation due to coalescence and Ostwald ripening, which 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 efficient forms of storage that may be needed in order to ensure the stability of the emulsion and the maintenance of urea coating. Phase separation could occur during storage however, it was not observed herein due to the almost immediate use of the materials after manufacturing.

When comparing the FA composition of the isolated and coated LTVF, a reduction in PUFA content of the vegetable fat was observed in response to the coating. Such reduction may be attributed to the interaction between urea and LTVF during the formation of the coating matrix, particularly due to the heating of the vegetable fat in an alkaline medium. At 60°C, the urea solution had a pH of 9.2. LTVF originates from the hydrogenation of soybean oil because 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 resulting from the coated material does not support evidence of augmentation of *trans* fatty acid content of the milk or meat. However, it is important to consider that this lipid profile can still undergo through additional modifications in the rumen due to the biohydrogenation promoted by rumen microorganisms (Larqué et al., 2003). Therefore, further studies are necessary to determine if the fatty acid composition of these SRU products can indeed affect milk and meat of animals fed diets containing this material. Nonetheless, the use of LTVF for urea coating was efficient.

When SRU was added to the diet, the EE content increased due to the addition of LTVF. As SRU inclusion levels increased, and soybean meal decreased, higher amounts of corn had to be added, then increasing dietary NFC, supporting the increase in NFC intake and apparent digestibility observed herein.

The addition of SRU to the diet increased the time spent eating and reduced the rumination time of the lambs. The LTVF used in the SRU probably promoted a coating of dietary fiber in the Tifton hay used as roughage, as the composition of the EE consumed increased, while decreasing NDF. The increased eating time confirms that SRU did not promote astringency in the lambs' consumption, which commonly occurs when uncoated urea is provided (Ribeiro et al., 2011; Mashayekhi et al., 2022).

Urea undergoes rapid hydrolysis in the rumen, releasing substantial amounts of NH_3-N and CO_2. This process can lead to nutrient desynchronization, specially the degradation of non-fiber and fibrous carbohydrates. Slow-release urea reduces abrupt hydrolysis and

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promotes better synchronization (Benedeti et al., 2014; Pacheco, 2021). According to Van Soest (1994), synchronizing protein degradation with carbohydrates in the rumen optimizes the utilization of rumen-degradable protein, thereby minimizing ammonia losses through the rumen wall. It is plausible that Slow-Release Urea (SRU) facilitates enhanced synchronization with dietary carbohydrates, resulting in improved nitrogen utilization, increased microbial protein production, and enhanced CP digestibility when compared to conventional urea.

SRU coated with LTVF improved N use by the lambs by reducing excretions and providing greater N ruminal retention - possibly attributed to SRU (de Medeiros et al., 2019; Netto et al., 2020; Melo et al., 2021), thereby resulting in a more balanced distribution of urea yields over time, and positively impacting ammonia utilization while reducing BUN. This constant NH_3-N production provided greater N availability to rumen microorganisms and despite the slightly reduction in rumen pH, it led to increase in the protozoa population. The increase in NH_3-N can also be attributed to a greater efficiency of U recycling, which is regulated by N-intake in growing ruminants. To facilitate this nitrogen transfer, the kidney and gastrointestinal tract can recover the excreted urea and redirect it to the gastrointestinal tract, where rumen bacteria make better use of nitrogen from BUN (Marini and Van Amburgh, 2003).

Kaneko et al. (2008), established physiological range values of BUN for sheep to be anywhere between 17 and 43 mg/dL. For lambs fed SRU_{40}, BUN values have always remained within this physiological range, even for the highest dietary level of coated urea (3 % of SRU_{40}), equivalent to 1.2 % inclusion (DM basis) of free urea. In contrast, the diet with free urea showed values that exceeded the physiological limit at time points 2, 4 and 6 h, but no animal showed clinical signs of intoxication during the experimental period.

The results demonstrated the efficiency of using LTVF as a urea coating agent, facilitating slow release and effectively reducing the urea hydrolysis peak. These findings align with thermal analyses, particularly the DSC analysis, indicating SRU40 as the most stable formulation. The improved stability led to a more consistent distribution of urea release over time, positively influencing ammonia utilization. This observation is consistent with the studies conducted by Geron et al. (2016) and de Medeiros et al. (2019)

The rumen wall will only absorb ammonia in the non-ionized form (NH_3). Lowering pH promotes ammonia ionization hence reducing ammonia uptake, whereas raising pH enhances ammonia uptake. Furthermore, the concentration of ammonia is influenced by the rates of ammonia input (diet) and output (blood serum). This phenomenon corroborates with the observed decrease in BUN, even with an increase in the amount of urea fed to the lambs (Nolan and Dobos, 2005; Kozloski, 2011).

The higher rumen pH observed in lambs fed uncoated urea is attributed to the faster hydrolysis of urea when fed in its free form, leading to an increased ammonia production. Nutritional factors, particularly readily fermentable carbohydrates such as starch, play a significant role in influencing rumen fluid pH (Alves et al., 2012). Variations in rumination time and the production of rumen fermentation products are among the outcomes of these factors. For optimal multiplication of rumen bacteria, the pH needs to be within the range of 5.5–7.0. This pH range ensures that microorganisms optimize diet utilization (Hoover and Stokes, 1991; Oliveira et al., 2019).

We hypothesize that SRU facilitated the utilization of NH_3-N by rumen microorganisms for bacterial protein synthesis, resulting in an augmented supply of both nitrogen and microbial protein. This hypothesis is supported by the N-metabolism, particularly in relation to the observed increase in N-retention and the subsequent decrease in its excretion through feces and urine (Jones and Milligan, 1975; Wang et al., 2018). N-retention is a key indicator of the body's ability to use and synthesize protein from available nitrogen (Makkar et al., 1981; Berends et al., 2014).

Possibly, this strategy enabled rumen microorganisms to optimize the utilization of ammonia for bacterial protein synthesis, leading to an increased microbial protein supply. This is supported by the increase in 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 herein showed that lambs fed SRU_{40} exhibited higher N-retention when compared to those fed $U_{0.5\ \%}$.

The enhanced efficiency in N metabolism not only benefits animal performance but also contributes positively to the environment. The reduction in N-excretion through feces and urine is important because excess N-excreted poses environmental risks, particularly when it accumulates in the soil and potentially contaminate water resources (Tamminga, 1996; Ridoutt et al., 2017). Therefore, using LTVF-coated urea, such as SRU, offers the advantage of mitigating environmental impacts associated with ruminant production systems.

Additionally, replacing soybean meal with coated urea without compromising the metabolism of lambs offers the potential for producers to have financial advantages. This includes reduction in feed costs without compromising animal performance as well as reduction of the impacts of the seasonal soybean market fluctuation on their production system.

The results obtained in this study demonstrate that SRU improves the efficiency of N utilization by ruminants, optimizes the use of dietary nitrogen and reduces losses of excreted nitrogen compounds, either through feces or urine. These outcomes have direct impact the in environment, specifically in reducing the contribution of N-excreted compounds to environmental pollution and reducing production costs of feeding operations.

The limitation of the material for industrial use arises from the melting point of the vegetable fat, which can cause phase separation of the emulsion at high temperatures, thereby reducing the efficacy of urea protection for slow release. This phenomenon compromises the emulsion stability, potentially resulting in inadequate urea release in the animals' rumen. Consequently, the ability to provide controlled and consistent urea release over time is compromised, negatively impacting process efficiency and final product efficacy. This limitation necessitates further investigation and alternative formulation strategies to overcome challenges associated with stability under different temperature and industrial processing conditions.

5. Conclusion

Low-trans vegetable fat demonstrated high efficiency in coating urea, particularly in the formulation containing 40 % urea and 60

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% vegetable fat (SRU_{40}). This formulation exhibited not only a higher yield and efficiency of coating but also greater thermal stability, highlighting its effectiveness in the urea coating process.

It is recommended to include 3 % SRU_{40} coated by LTVF in the diet of lambs since it enhances CP digestibility and the utilization of ammonia by rumen microorganisms, thereby reducing N-excretion. This is crucial from an environmental perspective as it improves N-balance and microbial protein production without negatively affecting performance.

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the study reported in this paper.

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Ethics approval for Animal use

All procedures followed the guidelines recommended by the National Ethical Committee for the Control of Animal Experimentation (CONCEA, Brazil) for the use of fistulated animals (Approval Protocol Number 059/2021).

Consent to publication

This manuscript is part of the academic thesis of the author Kevily Lucena defended in the Graduate Program in Science and Animal Health at the Federal University of Campina Grande. All authors consent to the publication of this manuscript.

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

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Thesis presented to the Doctoral Program in Animal Science, at the Federal University of Bahia as a partial requirement for the degree of Doctor in Animal Science.

Area of Concentration: Ruminant Production

Advisor: Prof. Dr. Ronaldo Lopes Oliveira
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SLOW-RELEASE UREA IN THE LAMB RUMEN BY MICROSPHERES VEGETABLE FAT

Pedro Henrique Soares Mazza

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SUMMARY

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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

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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

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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

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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.

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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

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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.

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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

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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.

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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.

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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

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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

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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.

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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

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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

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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)

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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

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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

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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}.

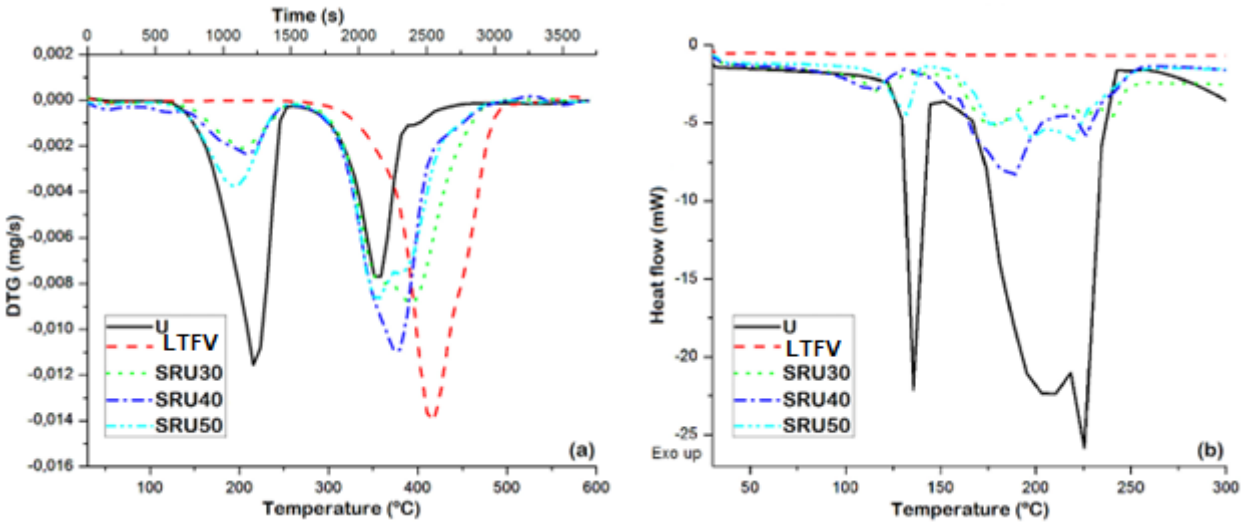

Figure 1. (a) Thermogravimetric curves (DTG curves) and (b) Differential scanning calorimetry curves (DSC curves) for free urea (U), low-*trans* vegetable fat (LTVF) and urea systems protected by vegetable fat (SRU_{30}, SRU_{40}, and SRU_{50}).

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.

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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.

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.

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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

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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).

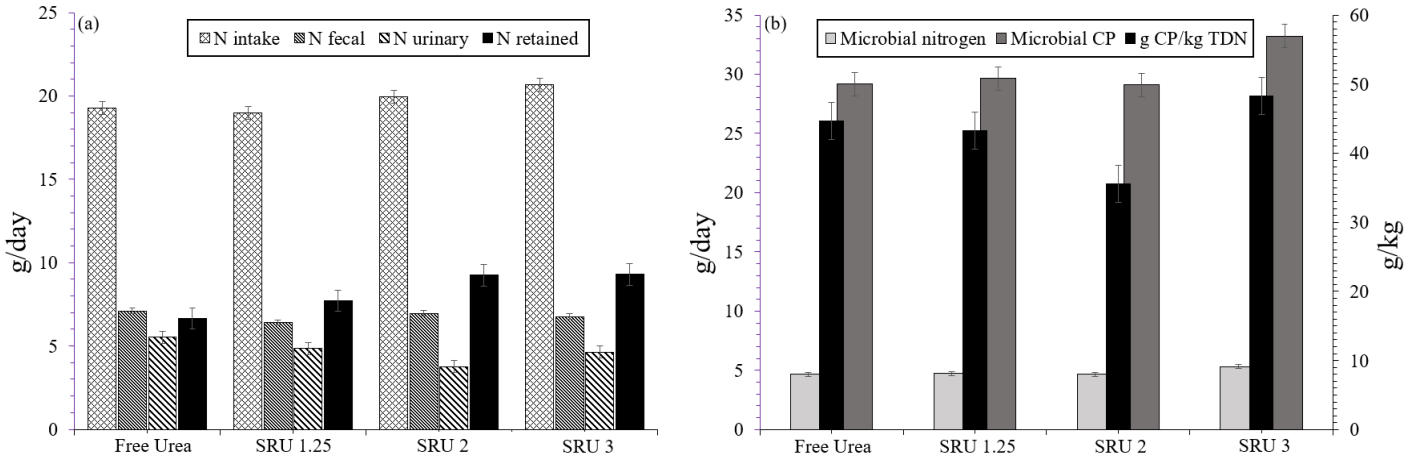

Figure 2. (a) Means of nitrogen (N) metabolism variables, N-intake, N-fecal and N-urinary excretion and N-retained (g/day); and (b) Means of microbial nitrogen and microbial protein production (g/day) and N-microbial production/TDN (energy) intake ratio in lambs (n = 32) fed slow-release urea (SRU40) 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 $U_{0.5}$

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(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.

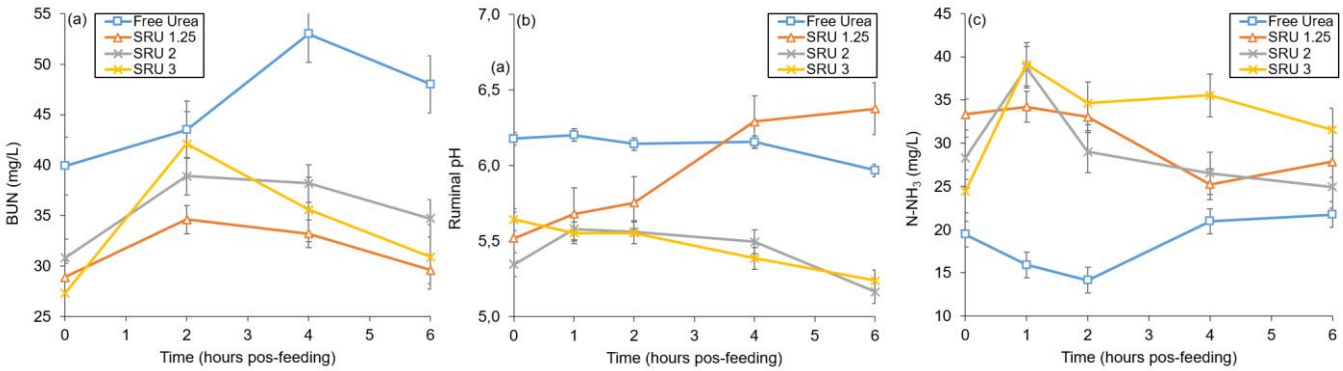

Figure 3. Means over hours post-feeding of blood urea nitrogen (BUN) (a) rumen pH (b) and ammonia nitrogen (N-NH3) (c) in lambs (n = 32) fed slow-release urea (SRU40) core protected by the low-trans vegetable fat matrix (40% of U: 60% of LTVF ratio) and added in 1.25% (SRU1.25), 2%(SRU2) and 3%(SRU3)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

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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

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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

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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).

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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

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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.

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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

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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.

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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

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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

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(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

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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

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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)				

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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*.

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(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

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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).

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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

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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

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= 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.

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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).

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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

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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

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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).

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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.

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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

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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}.

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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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RESUMO

UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS

A presente invenção engloba a obtenção de sistemas microencapsulados, através da microencapsulação de ureia utilizando gordura vegetal hidrogenada com baixo teor de trans como material encapsulante, pela técnica de Fusão (Melt), possibilitando aplicação das microesferas desenvolvidas na dieta de ruminantes. Esta tecnologia contorna as limitações do uso direto da ureia e permite sua liberação programada, proporcionando proteção frente ao ambiente ruminal e disponibilização controlada no sítio de interesse. Isso resulta na redução dos riscos de intoxicação por ureia e melhora o aproveitamento do nitrogênio pela microbiota ruminal, otimizando o uso da ureia como fonte de nitrogênio não proteico (NPN). A gordura vegetal hidrogenada constitui uma alternativa eficiente para microencapsular a ureia devido às suas características hidrofóbicas e estabilidade térmica, além de ser um produto acessível e de baixa degradabilidade. As formulações desenvolvidas melhoram a integridade da ureia durante o armazenamento e facilitam a homogeneização com os demais ingredientes da dieta, viabilizando a aplicação das micropartículas na dieta de ovinos e outros ruminantes. Além disso, essa técnica propicia menor excreção de nitrogênio no ambiente, contribuindo para a sustentabilidade ambiental da produção animal. As micropartículas de ureia encapsuladas com gordura vegetal hidrogenada permitem uma liberação gradual da ureia no rúmen, aumentando a eficiência de produção de proteína microbiana e melhorando o balanço de nitrogênio dos animais, sem comprometer a qualidade dos produtos cárneos.

UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS

CAMPO DA INVENÇÃO

[001] A presente invenção consiste na obtenção de ureia encapsulada em matriz lipídica de gordura vegetal com baixo teor de ácidos graxos trans, com o objetivo de desenvolver sistemas de liberação controlada para a alimentação de ruminantes. Esta tecnologia visa superar os desafios relacionados à aplicação direta da ureia na dieta desses animais. O produto desenvolvido através da microencapsulação de ureia com gordura vegetal permite sua incorporação eficaz na dieta de ruminantes. Isso melhora a estabilidade da ureia durante o armazenamento e facilita sua distribuição homogênea com outros ingredientes dietéticos, e no ambiente ruminal. Além de viabilizar a aplicação da utilização de maiores concentrações de ureia na dieta de ruminantes, esta tecnologia possibilita a obtenção de sistemas de liberação controlada de nitrogênio. Esses sistemas são projetados para resistir às condições degradativas no ambiente ruminal, garantindo uma disponibilização gradual e eficiente do nitrogênio no sítio de absorção, promovendo assim um aproveitamento nutricional mais eficaz. Adicionalmente, esta tecnologia contribui para uma utilização mais eficiente da ureia pelos ruminantes, reduzindo a excreção de nitrogênio no ambiente e melhorando a eficiência alimentar dos animais.

ANTECEDENTES DA INVENÇÃO

[002] Com os constantes avanços na produção animal, busca-se atualmente a inclusão de fontes alternativas de alimentos na dieta, com o intuito de proporcionar uma melhor economicidade das dietas e consequentemente um melhor valor agregado aos animais de produção, gerando um melhor produto final, sendo as fontes lipídicas associadas na proteção da ureia uma interessante opção.

[003] Por esta razão, há crescente valorização econômica de produtos que possam substituir o farelo de soja como fonte de proteína, promovendo uma maior economicidade das dietas formuladas para ruminantes.

[004] A gordura vegetal, especificamente a gordura vegetal de baixo teor de ácidos graxos trans, representa uma solução inovadora para a proteção e liberação controlada de ureia no rúmen. Devido ao seu processo de hidrogenação química, a gordura vegetal de baixo teor de ácidos graxos trans apresenta um ponto de fusão elevado, conferindo-lhe uma textura semi-sólida à temperatura ambiente, como evidenciado por Mazza *et al*. (2024). Essa característica é fundamental para o revestimento e encapsulamento eficaz de compostos como a ureia.

[005] A gordura vegetal de baixo teor de ácidos graxos trans oferece vantagens significativas sobre outros materiais de revestimento devido à sua estrutura físico-química. Sua textura semi-sólida proporciona uma liberação controlada e gradual da ureia no rúmen, o que é crucial para evitar picos excessivos de amônia nitrogênio e melhorar o balanço de nitrogênio nos animais, o que foi demonstrado no trabalho de Lucena *et al*. (2024), ao testar diferentes proporções de ureia e gordura vegetal no encapsulamento da ureia. Além disso, ao utilizar gordura vegetal de baixo teor de ácidos graxos trans, é possível melhorar o desempenho dos animais e reduzir a emissão de amônia para o meio ambiente (Lucena *et al*., 2024).

[006] A utilização de gordura vegetal de baixo teor de ácidos graxos trans como revestimento para ureia representa uma abordagem promissora para melhorar a eficiência nutricional e reduzir impactos ambientais na alimentação de ruminantes, aproveitando suas propriedades físico-químicas para um desempenho superior e sustentável.

[007] Utilizar fontes de nitrogênio não proteico (NPN), como a ureia, na dieta de ruminantes pode substituir parcialmente as fontes de proteína verdadeira quando usado em níveis adequados. No entanto, quando fornecido em quantidades elevadas, o NPN, como a ureia, pode causar intoxicação. Portanto, para reduzir os riscos e otimizar a utilização da ureia, pesquisadores

têm explorado métodos para revesti-la. Isso permite um aumento da dosagem diária e, consequentemente, uma utilização gradual pelos microrganismos do rúmen (Geron et al., 2016; Carvalho et al., 2019; de Medeiros et al., 2019; Melo et al., 2021), já que esses têm a capacidade de transformar fontes de NPN em proteína microbiana. Como a proteína microbiana representa uma fonte importante de aminoácidos para os ruminantes, maximizar a eficiência de sua produção pode, consequentemente, melhorar a produtividade (NRC, 2007).

[008] Para produzir ureia de liberação lenta (ULL) de alta qualidade, o agente de revestimento precisa ser inerte no rúmen, insolúvel em água e hidrofóbico. Essas propriedades garantem a capacidade do revestimento de prevenir a rápida liberação do núcleo, evitando o excesso de NH3-N no rúmen. de Medeiros et al. (2019) e Netto et al. (2021) testaram a cera de carnaúba como matriz de revestimento, enquanto Carvalho et al. (2019) testaram a cera de abelha. Eles demonstraram a viabilidade de revestir a ureia com uma matriz lipídica de ceras ricas em ácidos graxos saturados (SFA). Isso permite que a ureia seja liberada gradualmente no rúmen, reduzindo os riscos de toxicidade e aumentando a disponibilidade de NH3-N para que as bactérias produzam proteína microbiana de forma mais eficiente. Esses avanços têm o potencial de melhorar o balanço de nitrogênio e o desempenho animal.

OBJETIVO DA INVENÇÃO

[009] O objetivo da invenção é obter ureia microencapsulada em matriz lipídica de gordura vegetal hidrogenada pela técnica de fusão (Melt), possibilitando a busca de alternativas viáveis para solucionar dificuldades na incorporação de nitrogênio não proteico na dieta de ruminantes. Essa tecnologia visa melhorar a eficiência de utilização da ureia, permitindo sua liberação controlada no rúmen, reduzindo os riscos de toxicidade e aumentando a disponibilidade de nitrogênio para a síntese de proteína microbiana. Além disso, a invenção busca fortalecer o desenvolvimento de alimentos funcionais, protegendo a molécula de ureia e melhorando seu aproveitamento em condições específicas desejadas.

SUMÁRIO DA INVENÇÃO

[010] É objeto da presente invenção desenvolver sistemas microencapsulados de disponibilização programada, utilizando gordura vegetal hidrogenada como material encapsulante para ureia, através da técnica de fusão (Melt). O resultado será a produção de microesferas lipídicas contendo ureia, permitindo o manuseio no estado pastoso, maior resistência às condições do ambiente ruminal e liberação adequada no sítio de interesse, conforme as etapas a seguir:

a. Pesagem das respectivas massas de gordura vegetal com baixo teor de trans, ureia e lecitina;
b. Aquecimento da gordura vegetal com baixo teor de trans em banho maria a 60 ºC com 1% de lecitina de soja;
c. Solubilização da ureia em água na proporção 50:50 e aquecimento em banho maria a 60 ºC;
d. Incorporação e homogeneização da solução aquosa de ureia, lecitina e gordura vegetal com baixo teor de trans à 60 ºC;
e. Agitação contínua da emulsão obtida por 20 minutos;
f. Após a retirada do banho maria, deixar o material esfriar em temperatura ambiente;
g. Secagem em estufa de ventilação forçada por 24 horas a 55 ºC;
h. Homogeneização da pasta após a desidratação e armazenamento em refrigeração a 2ºC.

DESCRIÇÃO DETALHADA DA INVENÇÃO

[011] A presente invenção refere-se ao produto ureia de liberação lenta protegida com gordura vegetal hidrogenada com baixo teor de trans para liberação programada, utilizando gordura vegetal hidrogenada como material encapsulante e a técnica de Fusão (Melt), com proporções variadas entre encapsulante (gordura vegetal hidrogenada) e núcleos (ureia).

[012] O encapsulante lipídico usado, preferencialmente gordura vegetal hidrogenada, deve ser fundido preferencialmente em aquecimento indireto (banho maria) em temperatura que possibilite sua completa fusão.

[013] Para a formação da dispersão de gordura vegetal hidrogenada e ureia, a porcentagem de ureia deve ser preferencialmente de 40% em relação à massa total.

[014] Para a formação da fusão, a concentração de lecitina de soja deve ser preferencialmente 1% em relação à massa total da gordura vegetal hidrogenada.

[015] Após a fusão da gordura vegetal hidrogenada, a ureia deve ser adicionada lentamente, sob agitação contínua, até completa homogeneização.

[016] A dispersão entre núcleos e encapsulante deve ser em Banho Maria e adição de água destilada, sob agitação constante preferencialmente de 500 rpm, por 20 min.

[017] O excesso de água será removido preferencialmente por secagem em estufa.

[018] As microesferas lipídicas serão preferencialmente secas em estufa a 55 °C por 24 h, considerando ajustes destes parâmetros conforme encapsulante lipídico utilizado.

[019] Por fim, os sistemas microencapsulados serão homogeneizador para obtenção de uma pasta homogêneo, seguida de armazenamento em resfriamento em baixa temperatura, preferencialmente a 2ºC.

REIVINDICAÇÕES

1. UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS, **caracterizado por** compreender os seguintes componentes e etapas:

a. Pesagem das respectivas massas de gordura vegetal com baixo teor de trans, ureia e lecitina;
b. Aquecimento da gordura vegetal com baixo teor de trans em banho maria a 60 ºC com 1% de lecitina de soja;
c. Solubilização da ureia em água na proporção 50:50 e aquecimento em banho maria a 60 ºC;
d. Incorporação e homogeneização da solução aquosa de ureia, lecitina e gordura vegetal com baixo teor de trans à 60 ºC;
e. Agitação contínua da emulsão obtida por 20 minutos;
f. Após a retirada do banho maria, deixar o material esfriar em temperatura ambiente;
g. Secagem em estufa de ventilação forçada por 24 horas a 55 ºC;
h. Homogeneização da pasta após a desidratação e armazenamento em refrigeração a 2ºC.

2. PROCESSO DE OBTENÇÃO UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS, **caracterizado por** compreender preferencialmente a gordura vegetal com baixo teor de trans como lipídio encapsulante.

3. PROCESSO DE OBTENÇÃO UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS, **caracterizado por** compreender a porcentagem de ureia preferencialmente de 40% em relação à massa total e diluída em água na proporção de 50:50.

4. PROCESSO DE OBTENÇÃO UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS, **caracterizado por** compreender a lecitina de soja como agente emulsificante, com concentração preferencialmente de 1% em relação à massa total da gordura vegetal com baixo teor de trans.

5. PROCESSO DE OBTENÇÃO UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS, **caracterizado por** compreender temperatura que mantenha todos os materiais fundidos, preferencialmente 60 °C.

6. PROCESSO DE OBTENÇÃO UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS, **caracterizado por** compreender agitação mecânica constante por 20 min.

7. PROCESSO DE OBTENÇÃO UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS, **caracterizado por** compreender a remoção do excesso de água, preferencialmente por secagem em estufa a 55 ºC por 24h.

8. PROCESSO DE OBTENÇÃO UREIA DE LIBERAÇÃO LENTA PROTEGIDA COM GORDURA VEGETAL COM BAIXO TEOR DE TRANS, **caracterizado por** compreender a homogeneização dos sistemas microencapsulados para obtenção de uma pasta homogêneo, seguida de armazenamento em resfriamento em baixa temperatura, preferencialmente a 2ºC.