Animal Nutrition 7 (2021) 521–529

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Dietary supplementation with curcumin-loaded nanocapsules in lambs: Nanotechnology as a new tool for nutrition

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ARTICLE INFO

Article history:
Received 15 February 2020
Received in revised form
17 May 2020
Accepted 2 June 2020
Available online 17 March 2021

Keywords:
Antioxidant
Anti-inflammatory
Curcuma longa
Performance
Sheep

ABSTRACT

Curcumin-containing nanocapsule powder formulations have not been used in ruminant feed to date, despite the fact that curcumin is known to be a functional food additive. The objective of this study was to determine whether ethyl polymethacrylate (Eudragit L-100) nanocapsules loaded with curcumin (N-CU) would improve health and growth of lambs. Thirty-two male Lacaune lambs (body weight [BW] = 16 ± 0.99 kg; 45 d of age) were randomly assigned to 1 of 4 treatments: T0, T1, T2 and T4, representing supplementation of curcumin at 0, 1, 2, and 4 mg/kg concentrate, respectively. The animals in each treatment were allocated in 4 pens of 2 lambs each (8 lambs per treatment). The experiment lasted 17 d, with samples and measurements collected on d 0, 7, 12, and 17. The T2 lambs had greater average daily gain than T0 lambs. Regression analysis showed that the ideal dose of N-CU to enhance weight gain was 1.89 mg/kg concentrate. There were significant interactions ($P < 0.05$) between treatments × time for hematological variables, particularly for increases in erythrocytes (T2) and reductions in counts of leukocytes, neutrophils, and lymphocytes in T1 and T2. There were significant interactions between treatment × time for total protein, globulin, urea, and triglyceride levels. Stimulation of the antioxidant system was also observed. There were increased levels of non-protein thiols (NPSH), as well as increased activities of superoxide dismutase (SOD) and glutathione S-transferase (GST) in the supplemented animals. Levels of reactive oxygen species (ROS) were lower in the serum of supplemented lambs. In general, the 4 mg/kg dose had no positive effects on growth or health. This was an unexpected result, given the known properties of curcumin. Taken together, these findings suggest that addition of low concentrations of nanoencapsulated curcumin (T1 and T2) in lamb feed improves health, minimizing oxidative stress and generates anti-inflammatory effects that may have contributed indirectly to greater weight gain. Nanocapsules potentiate the effects of curcumin and may emerge as a new tool in animal nutrition.

1. Introduction

Care for lambs from birth to weaning is crucial to the success of sheep farming because such care maximizes weight and determines the age of weaning. For early weaning of lambs between 45 and 60 d of age, it is necessary to provide supplements that produce higher performance and better health, in addition to promoting adequate ruminal development and helping the animal consume solid feed (Slukwa, 2014; Zhang et al., 2019). Nursing

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2020.06.014

lambs that have higher feed efficiency will have better performance; however, to accomplish this goal, it is important that the lambs eat quality solid feed, coupled with less milk to facilitate early weaning (Ciria et al., 2009).

Natural additives promoting animal health have been studied, including curcumin, i.e. (1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione, an herbal compound derived from *Curcuma longa* (Sueth-Santiago et al., 2015). Curcumin has anti-inflammatory properties, including inhibition of tumor necrosis factor alpha (TNF-α) and interleukins (IL-1 and IL-6) (Liu et al., 2015), all of which are proinflammatory cytokines' actions capable of stimulating the production and activation of macrophages and monocytes (de Oliveira et al., 2011). Curcumin has also antioxidant properties such that it donates electrons and possesses phenol moieties that capture free radicals, thereby minimizing cell damage in animal organism (Sueth-Santiago et al., 2015).

Curcumin has been used in animal production, mainly in chicken feed (Salah et al., 2019; Adegoke et al., 2018) and ruminant feed (Jaguezeski et al., 2018; Molosse et al. al. 2019). Researchers who fed sheep curcumin found increased milk yield and animal health benefits resulting from antioxidant, anti-inflammatory, and hepatoprotective effects (Jaguezeski et al., 2018). Molosse et al. (2019) found greater weight gain in lambs fed free curcumin.

Curcumin in its natural form has some disadvantages, including low water solubility, low availability, rapid elimination (Wang et al., 2009), high instability at acid or alkaline pH, high light sensitivity and rapid degradability (Joung et al., 2016). Methods designed to improve the absorption of bioactive components and to slow their release have been reported (Pereira et al., 2010). Ethyl polymethacrylate nanocapsules are easily encapsulated, expandable, and acid-stable (Pereira et al., 2010). This nanotechnology aims to improve curcumin absorption at the intestinal level, reducing the dose in feed, with slow release of the active ingredient, especially at pH above 6.0, that is, in the ruminant gut (Pereira et al., 2010; Jaguezeski et al., 2019; Beloqui et al., 2014; Manjili et al., 2017; Yao et al., 2017). Jaguezeski et al. (2019) supplemented sheep with ethyl polymethacrylate (Eudragit L-100) nanocapsules loaded with curcumin (N-CU) and found that the preparation had antioxidant effects in whey and milk, thereby reducing potentially harmful oxidative reactions.

Proper feeding of lambs is crucial for efficient production. At the end of the production cycle, the goal is to produce larger and healthier animals that are more profitable for the farmer. The results of Jaguezeski et al. (2018; 2019) and Molosse et al. (2019) led us to hypothesize that Eudragit L-100 nanocapsules containing curcumin would potentiate the positive effects of curcumin in ruminant diets. Therefore, the aim of this study was to determine whether addition of low-concentration of N-CU would improve health and growth of lambs.

2. Materials and methods

2.1. Ethics committee

This project was approved by the Committee for the Use of Animals in Research (CEUA) of the Santa Catarina State University (UDESC) under protocol number 1962030419. It complied with the rules issued by the National Council for Control of Animal Experimentation (CONCEA).

2.2. Preparation of nanocapsules containing curcumin

Curcumin was purchased from Sigma–Aldrich. The product has a 99% purity guarantee. Curcumin-loaded ethyl polymethacrylate (Eudragit L-100) nanocapsules were prepared and characterized at the Nanotechnology Laboratory of the Franciscan University (Santa Maria, RS, Brazil). Curcumin-loaded nanocapsules (0.25 mg/mL) were prepared using the preformed polymer interfacial deposition method (Jaguezeski et al., 2019). The nanocapsules presented an average size 136 ± 0.96 nm, polydispersion index 0.11 ± 0.01, zeta potential −18.0 ± 1.24 mV, pH 4.27 ± 0.02 and curcumin content (97 ± 1)%. Spray dried powder containing nanoencapsulated curcumin was obtained using a spray-drying technique with lactose as an adjuvant (Ourique et al., 2014). The drying process had a yield of (87.0 ± 2.3)%, with mean particle size after redispersion 175 ± 1.2 nm, polydispersion index 0.16 ± 0.02, zeta potential −16 ± 0.78 mV, pH 4.43 ± 0.04 and curcumin content (90 ± 1)%. At the end of the process, HPLC analysis showed that the N-CU powder contained curcumin at 2.0 mg/g.

2.3. Animals, and experimental design

Thirty-two male Lacaune lambs with average weight 16 ± 0.99 kg and 45 d of age were used in the study. Lambs were randomly assigned to 1 of 4 treatments: T0, T1, T2, and T4, representing supplementation of N-CU at 0, 1, 2, and 4 mg/kg concentrate, respectively. The animals in each treatment were allocated in 4 pens with 2 lambs each (8 lambs/treatment). Knowing that every 1 g of N-CU powder contained 2 mg of curcumin, the doses were calculated and added to the concentrate. Because the adjuvant of the N-CU formulation was lactose, the amount of lactose was calculated and mixed in the concentrate of the treatments T0, T1, and T2. The doses used in the current study were defined based on the experiment of Jaguezeski et al. (2019).

According to the number of animals available for study, we tested 3 levels of nanostructured curcumin in the lamb diet. The effects of free curcumin on Lacaune lamb were already known at this stage (Molosse et al., 2019); this explains why a group of lambs receiving free curcumin was not included in this study (Jaguezeski et al., 2019).

Between 45 and 62 d of age, the experimental period, the animals received milk once a day (morning), a volume of 500 mL per day per animal. Water was provided ad libitum.

The base concentrate was formulated based on ground corn, soybean meal and premix (Table 1). The base concentrate was then supplemented with various concentrations of powdered N-CU. In the first week (7 d; adaptation period), each animal received 300 g of concentrate daily. This amount was increased to 400 g of concentrate per animal per day from d 8 to 17 of the experiment. The concentrate was offered to the animals twice a day (07:00, and 17:00), which was consumed in its entirety (100%) within 15 min after being made available; and in the sequence, the silage was offered.

Corn silage was supplied ad libitum, divided into 3 times of the day its replacement in the feeder (07:15, 12:00, and 17:15). Between

Table 1
Ingredients used to formulate the base concentrate of lambs (%, as-fed basis).

Item	Content
Ground corn	59.0
Soybean meal	37.0
Premix[1]	4.00
Total	100

[1] Premix composition: phosphorus, ≥55 g/kg; calcium, ≥215 g/kg, ≤225 g/kg; sulfur, ≥12 g/kg; sodium, ≥80 g/kg; cobalt, ≥60 mg/kg; chromium, ≥12 mg/kg; iron, ≥1,420 mg/kg; iodine, ≥100 mg/kg; magnesium, ≥14 mg/kg; manganese, ≥1,550 mg/kg; selenium, ≥22 mg/kg; vitamin A, ≥20,000 IU/kg; vitamin D, ≥40,000 IU/kg; vitamin E, ≥550 IU/kg; and fluorine, ≤550 mg/kg.

d 8 and 17, the amount of silage available per pen/repetition was measured; the excess silage is weighed. Based on this information, the amount of feed consumed was calculated.

Concentrate and silage samples were collected and the chemical composition was further analyzed. Results are presented in Table 2.

A total of 0.5 g of concentrate samples were used for preparation and obtaining the homogenate in order to determine antioxidant activity by elimination of radicals by diphenyl picrylhydrazyl (DPPH) according to methodology described in detail by Alba et al. (2019). Activity was presented by the half maximal inhibitory concentration (IC_{50}) value (μg/mL), defined as the concentration of antioxidant required to eliminate 50% of the DPPH present in the test solution. All tests were performed in triplicate.

2.4. Body weight

Lambs were weighed using a digital scale, always at the same time (06:30), before the morning feeding (12 h fasting). The animals were weighed individually; however, for statistical analysis, the mean of 2 animals was considered as the body weight of the pen/repetition. The data were used to calculate the weight gain and average daily gain (ADG) at 4 time points (d 0, 7, 12, and 17).

2.5. Collection and processing of blood and feces samples

Blood was collected from the jugular vein on experimental d 0, 7, 12 and 17. Fecal samples were collected directly from the rectal ampulla (d 0 and 17) and stored on ice until analyses (same day that were collected). Total blood was collected in 2 tubes: without anticoagulant to obtain serum, and with EDTA as the anticoagulant for hemogram. The blood collected without anticoagulant was centrifuged at 870×*g* for 10 min, and the serum was stored at −20 °C until analysis. The tubes with EDTA were sent to the laboratory without centrifugation and the hemogram was analyzed (same day that blood was collected) in the whole blood samples.

2.6. Laboratory analysis

The chemical composition of concentrate and silage were analyzed according to AOAC (2000): dry matter (DM), method 930.15; crude protein (CP), method 976.05; ether extract (EE), method 920.39; and ash, method 942.05. The concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was measured according to the methodology of Van Soest et al. (1991) without the addition of sodium sulfite and alpha-amylase.

For hemogram analyses, the erythrocyte and total leukocyte counts, as well as hemoglobin (Hb) content were measured using a semi-automated analyzer (Celm 530). Hematocrit was obtained after capillary centrifugation (1,000 × *g* for 5 min). Leukocyte differential counts were performed in blood smears stained with commercial dye (*Panótico Rapido*) using a light microscope at 1,000 × magnification (Feldman et al., 2000).

Serum levels of total protein (TP), albumin, urea, glucose, triglycerides, aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) were measured on a semi-automated analyzer (BioPlus, 2000, Bioplus Produtos para Laboratórios Ltda, Baruiri − SP, Brazil) using specific commercial kits, a colorimetric method (Analisa, Gold Analisa Diagnóstica Ltda, Belo Horizonte − MG, Brazil) and following the manufacturer's recommendations. Globulin levels were calculated as total protein − albumin; according to literature (Kaneko et al., 2008).

Serum reactive oxygen species (ROS) levels were determined using the fluorescent dichloro fluorescein (DCF) oxidation method described by Lebel et al. (1992) with excitation and emission wavelengths of 485 and 538 nm, respectively. Results were expressed as units of DCFper milligram of protein.

Lipoperoxidation (LPO) levels were measured using serum samples diluted in cold methanol (1:1; vol/vol) and centrifuged at 1,000 × *g* for 10 min at 4 °C (Monserrat et al., 2003; da Silva Barreto et al., 2018). LPO levels were measured in the serum using a microplate reader at 550 nm, with cumene hydroperoxide as the standard.

Levels of non-protein thiols (NPSH) in serum were evaluated according to Sedlak and Lindsay (1968). The results were expressed as micro moles of sulfhydryl per milligram of protein.

Serum glutathione S-transferase (GST) activity was measured based on the method described by Habig et al. (1974). Enzymatic activity was expressed as units of GST per milligram of protein. The superoxide dismutase (SOD) activity in plasma was evaluated spectrophotometrically as described by Marklund and Marklund (1974). Enzymatic activity was expressed as uints of SOD per milligram of protein.

Parasitological examination was performed using the McMaster technique described by Gordon and Whitlock (1939) with saturated sugar solution to quantify the number of eggs per gram of feces (EPG).

2.7. Statistical analyses

All dependent variables were tested for normality using the Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) and were log-transformed when needed. Then, all data were analyzed using the MIXED procedure of SAS, with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The ADG was tested for fixed effect of treatment using pen (treatment) and animal (pen) as random effects. All other variables were analyzed as repeated measures and were tested for fixed effects of treatment, day, and treatment × day, using pen (treatment) and animal (pen) as random variables. The covariance structures were selected according to the lowest Akaike information criterion. Only the weight gain data were subjected to regression analysis to determine the ideal curcumin concentration for lamb diets, and were tested for linear, quadratic and root effects. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and $P \leq 0.10$.

3. Results

Concentrates from treatments T2 and T4 consumed by lambs had lower IC_{50} activity (Table 2), meaning these concentrates had higher antioxidant activity; this was consistent with treatments supplemented with higher levels of curcumin.

3.1. Growth and feed intake

The T2 lambs had greater ($P = 0.05$) ADG compared to T0 lambs (Table 3). The addition of N-CU to the lambs' diets showed a quadratic effect for weight gain, and regression analysis revealed that 1.89 mg of curcumin present in N-CU powder per kilogram of concentrate was optimal for increasing weight gain (Fig. 1).

Concentrate intake was 100% of that offered; as well as there was no difference between groups in silage consumption (Table 3). The silage consumption of all treatments was approximately 92%.

Table 2
Chemical composition of diets, and antioxidant activity of diphenyl picrylhydrazyl in concentrates.

Item	Silage	Treatments [1]			
		T0	T1	T2	T4
Chemical composition, %					
DM	33.7	89.6	90.1	90.3	89.8
Ash	4.1	3.4	3.8	3.5	2.9
Crude protein	8.1	19.1	18.5	19.4	18.7
EE	4.2	3.8	3.3	3.9	3.9
ADF	17.3	5.3	6.1	5.6	4.7
NDF	33.7	12.5	10.6	12.2	9.8
Antioxidant activity of diphenyl picrylhydrazyl					
IC_{50}, μg/mL	–	2.16 ± 0.001[a]	2.03 ± 0.27[a]	1.15 ± 0.25[b]	1.21 ± 0.31[b]

DM = dry matter; EE = ether extract; ADF = acid detergent fiber; NDF = neutral detergent fiber; IC_{50} = half maximal inhibitory concentration.
[a, b] Within a row, means without out a common superscript differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$) between treatments.
[1] T0, T1, T2 and T4 represents supplementation of polymethacrylate (Eudragit L-100) nanocapsules loaded with curcumin (N-CU) at 0, 1, 2 and 4 mg/kg concentrate, respectively.

3.2. Hematological analyses

Effects of treatment × day ($P = 0.05$) and treatment ($P = 0.03$) were detected for blood concentration of erythrocytes. T2 lambs had greater concentrations and T1 lambs had lower concentrations on d 12 and 17 than did T0 lambs (Table 4). Effects of treatment × day ($P < 0.01$) and treatment ($P = 0.05$) were detected for counts of leukocytes. T1, T2, and T4 lambs had lower counts on d 7, but T4 lambs had greater counts on d 17 than did T0 lambs (Table 4). Effects of treatment × day ($P < 0.01$) and treatment ($P = 0.02$) were detected for counts neutrophils. Compared with T0 lambs, T1, T2, and T4 lambs had lower counts on d 7, and T1 and T4 lambs had lower counts on d 12, and T4 lambs had greater counts on d 17 (Table 4). Effects of treatment × day ($P = 0.04$) but not treatment ($P = 0.33$) were detected for counts of lymphocytes, and T1 and T2 lambs had lower counts on d 17 than did T0 lambs (Table 4).

3.3. Serum clinical biochemistry

No effects of treatment × day ($P = 0.68$) but effects of treatment ($P = 0.09$) tended to be detected for serum concentration of AST, and T2 and T4 lambs had greater concentrations than did T0 lambs (Table 5). Effects of treatment × day ($P = 0.07$) and treatment ($P = 0.09$) tended to be detected for serum concentrations of GGT; T1 and T4 lambs had greater concentrations on d 7, and T4 lambs had greater concentrations on d 12 than did T0 lambs (Table 5). Effects of treatment × day ($P < 0.01$) and treatment ($P < 0.01$) were detected for total serum protein, and T4 lambs had lower concentrations on d 7, and T1, T2, and T4 on d 12, and T1 lambs on d 17, than did T0 lambs (Table 5). Effects of treatment × day ($P = 0.08$), but not treatment ($P = 0.64$) tended to be detected for serum concentrations of albumin, and T1, T2, and T4 lambs had lower concentrations on d 7, and T2 lambs had lower concentrations on d 12 than did T0 lambs (Table 5). Effects of treatment × day ($P = 0.01$) and treatment were ($P = 0.01$) detected for serum concentrations of globulin, and T1 lambs had lower concentrations on d 7, T1 lambs on d 12, and T1 and T2 lambs on d 17 than did T0 lambs (Table 5). Effects of treatment × day ($P = 0.09$), but not treatment ($P = 0.12$) tended to be detected for serum concentration of glucose, and T2 and T4 lambs on d 12, and T1 and T2 lambs on d 17, had lower concentrations than did T0 lambs (Table 5). Effects of treatment × day ($P = 0.04$), but not treatment ($P = 0.43$) were detected for serum concentration of triglycerides, and T1, T2 and T4 lambs had lower concentration on d 7, than did T0 lambs (Table 5). Effects of treatment × day ($P = 0.02$), but not treatment ($P = 0.84$) were detected for serum concentration of urea, and T4 lambs on d 7, and T1, T2 and T4 lambs on d 17, had greater concentrations than did T0 lambs (Table 5).

Table 3
Body weight, daily weight gain and feed intake of lambs fed curcumin nanocapsules.

Item	Treatments[1]				SEM	*P*-value	
	T0	T1	T2	T4		Treat	Treat × day
Body weight, kg						0.16	0.46
d 0	15.8	15.8	15.8	15.8	0.20		
d 7	17.4	17.9	18.1	17.7	0.20		
d 12	19.1	19.4	19.7	19.1	0.20		
d 17	20.4	20.8	21.2	20.6	0.20		
Average daily gain, kg/d							
d 0 to 7	0.224	0.298	0.324	0.273	0.02	0.30	
d 0 to 12	0.268	0.299	0.322	0.272	0.02	0.18	
d 0 to 17	0.269[b]	0.292[ab]	0.316[a]	0.281[ab]	0.01	0.05	
Concentrate intake, g/d							
d 0 to 7	300	300	300	300	0.00	–	
d 8 to 17	400	400	400	400	0.00	–	
Silage intake[2] (DM), g/d							
d 8 to 17	356	364	346	359	2.32	0.83	

[a, b] Within a row, means without out a common superscript differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$) between treatments.
[1] T0, T1, T2 and T4 represents supplementation of polymethacrylate (Eudragit L-100) nanocapsules loaded with curcumin (N-CU) at 0, 1, 2 and 4 mg/kg concentrate, respectively.
[2] During the adaptation period, silage consumption was not measured.

3.4. Serum oxidant/antioxidant status

Effects of treatment × day ($P > 0.05$) and treatment ($P > 0.05$) were not detected for serum concentration of LPO (Fig. 2A). Effects of treatment × day ($P < 0.01$), and treatment ($P = 0.02$) were detected for serum concentration of ROS, and T1, T2 and T4 lambs had greater concentrations on d 7, T2 lambs had lower concentrations on d 12, and T1, T2 and T4 lambs had lower concentration on d 17, than did T0 lambs (Fig. 2B). Effects of treatment × day were detected ($P < 0.01$) and treatment tended to be detected ($P < 0.10$) for serum NPSH (Fig. 2C), and T1 lambs had greater concentrations on d 7, and T1, T2 and T4 had greater concentrations on d 17, than did T0 lambs. Effects of treatment × day were detected ($P < 0.01$) and treatment tended to be detected ($P = 0.09$) for serum SOD, and T1, T2 and T4 had greater concentrations on d 17 than did T0 lambs (Fig. 2 D). Effects of treatment × day were detected ($P < 0.01$) and treatment tended to be detected ($P < 0.10$) for serum GST (Fig. 2E), and T1 lambs had greater concentrations on d 7, and T1, T2 and T4 lambs had greater concentrations on d 17 than did T0 lambs.

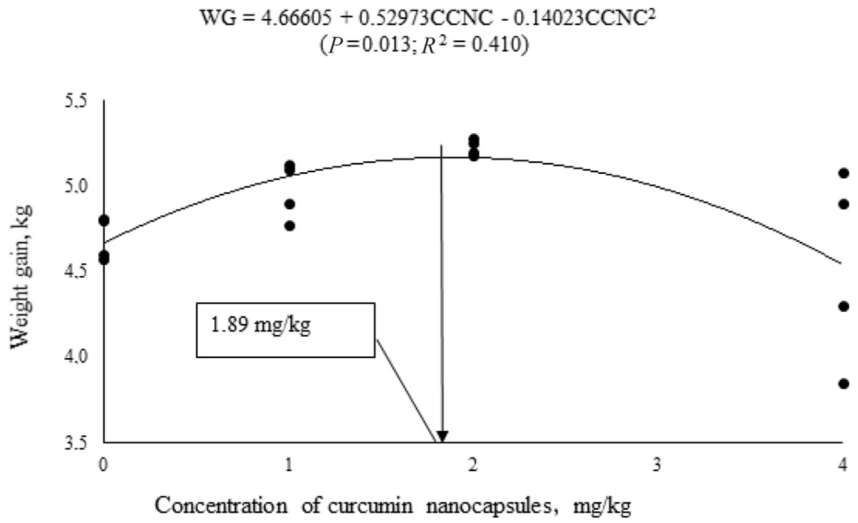

Fig. 1. Curve, equation and inflection point obtained via regression analysis of weight gain (d 0 to 17) in lambs fed curcumin nanocapsules. WG = weight gain; CCNC = concentration of curcumin nanocapsules in the concentrate.

Table 4
Hematological variables of lambs supplemented with curcumin.

Item	Treatments[1]				SEM	*P*-value	
	T0	T1	T2	T4		Treat	Treat × day
Erythrocytes, × 10^6 μL						0.03	0.05
d 0	8.49	8.29	7.26	7.81	0.62		
d 7	6.61	5.88	7.08	6.47	0.62		
d 12	6.20^{c}	8.36^{b}	10.3^{a}	7.94^{b}	0.66		
d 17	6.56^{b}	7.57^{ab}	9.41^{a}	6.98^{b}	0.62		
Hematocrit, %	35.2	33.9	34.7	34.6	0.48	0.30	0.21
Hemoglobin, g/dL	11.3	10.7	11.1	11.2	0.19	0.13	0.22
Leukocytes, × 10^3/μL						0.05	<0.0001
d 0	10.2	9.98	9.56	8.58	0.60		
d 7	9.34^{a}	7.44^{b}	7.18^{b}	7.40^{b}	0.60		
d 12	6.57^{a}	5.47^{c}	5.85^{ab}	6.02^{ab}	0.60		
d 17	6.78^{b}	5.20^{c}	6.09^{bc}	9.92^{a}	0.60		
Neutrophils, × 10^3/μL						0.02	<0.0001
d 0	3.66	3.28	3.11	3.08	0.31		
d 7	3.99^{a}	2.40^{b}	2.37^{b}	2.00^{b}	0.31		
d 12	2.87^{a}	1.91^{b}	2.29^{ab}	1.95^{b}	0.35		
d 17	2.53^{b}	2.16^{b}	2.56^{b}	4.59^{a}	0.31		
Lymphocytes, × 10^3/μL						0.33	0.04
d 0	5.64	6.23	6.12	5.16	0.43		
d 7	5.39	4.81	4.51	5.11	0.43		
d 12	3.52	3.28	2.70	3.60	0.49		
d 17	4.75^{a}	2.89^{b}	3.17^{b}	4.90^{a}	0.43		
Monocytes, × 10^3/μL	0.12	0.11	0.11	0.13	0.94	0.94	0.71
Eosinophils, × 10^3/μL	0.09	0.09	0.10	0.09	0.02	0.97	0.84

$^{a, b, c}$ Within a row, means without out a common superscript differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$) between treatments.
[1] T0, T1, T2 and T4 represents supplementation of polymethacrylate (Eudragit L-100) nanocapsules loaded with curcumin (N-CU) at 0, 1, 2 and 4 mg/kg concentrate, respectively.

3.5. Parasitological exams

No parasitic gastrointestinal infections were observed during the experimental period in all lambs. Parasitological examinations were negative for parasite eggs, cysts and oocysts.

4. Discussion

Curcumin-loaded Eudragit L100 nanocapsules facilitate intestinal absorption (Jaguezeski et al., 2019). However, for ruminants, this is challenge, given ruminal pH (5.5 to 7.2) (Santana Neto et al., 2012). Pereira et al. (2010) found that Eudragit L-100 nanocapsules opened in the digestive tract at pH 6 or higher. The present study was the first study to propose the use of spray dried curcumin-loaded nanocapsules in lamb feed; however, a formulation was previously used as a suspension by our low-dose curcumin research group (Jaguezeski et al., 2018). In both studies, antioxidant system stimulation and leukocyte reduction were described, as in the present study, where we observed weight gain in lambs, but no effect on feed consumption. Among the advantages of offering solid concentrate additives (N-CU powder) in animal feed are the stability, ease of preparation, and presence

Table 5
Serum biochemistry of lambs supplemented with curcumin nanocapsules.

Item	Treatments[1]				SEM	*P*-value	
	T0	T1	T2	T4		Treat	Treat × day
AST, U/L	113[b]	119[ab]	134[a]	134[a]	6.31	0.09	0.68
GGT, U/L						0.09	0.07
d 0	101	98.3	102	102	5.75		
d 7	96.2[b]	112[a]	95.6[b]	115[a]	5.75		
d 12	113[b]	116[b]	103[b]	132[a]	5.75		
d 17	121	114	112	113	5.75		
Protein total, g/dL						<0.0001	0.0003
d 0	5.25	5.61	5.26	5.34	0.31		
d 7	6.84[b]	5.90[c]	6.23[b]	6.99[a]	0.31		
d 12	6.46[a]	5.01[b]	5.54[b]	5.58[b]	0.31		
d 17	5.44[a]	4.34[b]	5.09[ab]	5.20[ab]	0.31		
Albumin, g/dL						0.64	0.08
d 0	2.78	3.18	3.23	2.96	0.21		
d 7	4.03[a]	3.34[bc]	3.12[c]	3.65[b]	0.21		
d 12	2.63[a]	2.49[ab]	2.14[b]	2.53[ab]	0.21		
d 17	3.20	3.64	3.62	3.60	0.21		
Globulin, g/dL						0.01	0.01
d 0	2.40	2.48	2.05	2.36	0.34		
d 7	2.75[a]	1.61[b]	3.13[a]	3.33[a]	0.34		
d 12	3.75[a]	2.57[b]	3.44[ab]	3.03[ab]	0.34		
d 17	2.17[a]	1.26[b]	1.51[b]	1.60[ab]	0.34		
Glucose, mg/dL						0.12	0.07
d 0	90.8	88.3	98.5	88.8	4.57		
d 7	86.6	88.2	85.5	79.3	4.57		
d 12	78.6[a]	71.0[ab]	65.4[b]	64.6[b]	4.57		
d 17	78.0[ab]	81.3[a]	58.6[c]	68.1[bc]	4.58		
Triglycerides, mg/dL						0.43	0.04
d 0	59.8	46.4	52.6	56.0	4.68		
d 7	48.1[b]	63.2[a]	69.6[a]	67.2[a]	4.68		
d 12	36.9	34.4	32.3	38.3	4.68		
d 17	41.9	51.7	45.3	49.6	4.68		
Urea, mg/dL						0.84	0.02
d 0	29.0	27.0	25.7	22.0	3.05		
d 7	25.6[b]	31.8[ab]	31.0[ab]	34.1[a]	3.05		
d 12	37.0	34.1	37.1	35.1	3.05		
d 17	33.9[b]	38.7[a]	43.1[a]	42.3[a]	3.05		

AST = aspartate aminotransferase; GGT = gamma glutamyltransferase.
[a-c] Within a row, means without out a common superscript differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$) between treatments.
[1] T0, T1, T2 and T4 represents supplementation of polymethacrylate (Eudragit L-100) nanocapsules loaded with curcumin (N-CU) at 0, 1, 2 and 4 mg/kg concentrate, respectively.

of lactose in the formulation to stimulate consumption. Because curcumin has low digestibility, nanoencapsulation is advantageous because it allows the addition of low doses, because nanocapsules only release curcumin in the intestine in a pH-dependent manner.

The greatest ADG in N-CU-fed lambs was related to the antioxidant and anti-inflammatory properties of the curcumin as well as by stimulation of erythrocyte production. In agreement with our observations, Molosse et al. (2019) found that lambs supplemented with free curcumin at 100 and 200 mg/kg over 30 d presented higher weight gain compared to an unsupplemented group, suggesting that these effects are also mediated by improvement in creatine kinase activity, an essential enzyme that generates an efficient temporal energy buffer and prevents decreases in ATP levels. Reduction of inflammatory responses allows reduced energy expenditure required to activate these defense mechanisms. Consequently, more energy remains to enhance the growth performance. The anti-inflammatory action of curcumin is well-described, as is its ability to reduce blood lymphocytes counts (Jaguezeski et al., 2019) that consequently lower serum globulin levels. This anti-inflammatory effect of curcumin has been attributed to its ability to inhibit IL-1, the cytokine primarily responsible for stimulating lymphocyte production (Sharma et al., 2007; Jaguezeski et al., 2019; Witko-Sarsat et al., 2011). It should also be noted that there is an apoptotic effect of curcumin on neutrophils (Colotta et al., 1998) that possibly explains the lower numbers of blood neutrophils in supplemented lambs.

In the current study, low doses of curcumin powder increased the antioxidant response. The mechanisms of curcumin's stimulation antioxidant activity are well known (Zhang et al., 2018; Rauf et al., 2018), as has been reported in lactating lambs (Molosse et al., 2019), dairy sheep (Jaguezeski et al., 2019), in laying hens and quails (Rajput et al., 2013; Marchiori et al., 2019), and in several studies with broilers already mentioned. Curcumin has phenolic moieties that capture free radicals, consequently reducing cell damage (Tapia et al., 2012). Moreover, curcumin stimulates the enzyme antioxidant system, thereby increasing the activities of enzymes such as SOD and GST (Cardozo et al., 2013), similar to what was observed in the current study. The increase in NPSH in lamb serum is also explained by the ingestion of curcumin. We found that intake of low levels of N-CU by lambs was sufficient to increase antioxidant activity. In agreement with our observations, Molosse et al. (2019) observed a significant increase on total antioxidant capacity in serum of lambs supplemented with curcumin at 100 and 200 mg/kg of diet on 15 and 30 d, as well as a reduction on serum lipid damage on d 30 post supplementation with curcumin at 200 mg/kg diet, revealing the potent antioxidant effects of free curcumin to lambs.

Lower serum glucose levels were observed in T2 and T4 animals. Curcumin increased glycerol kinase enzyme activity, consequently

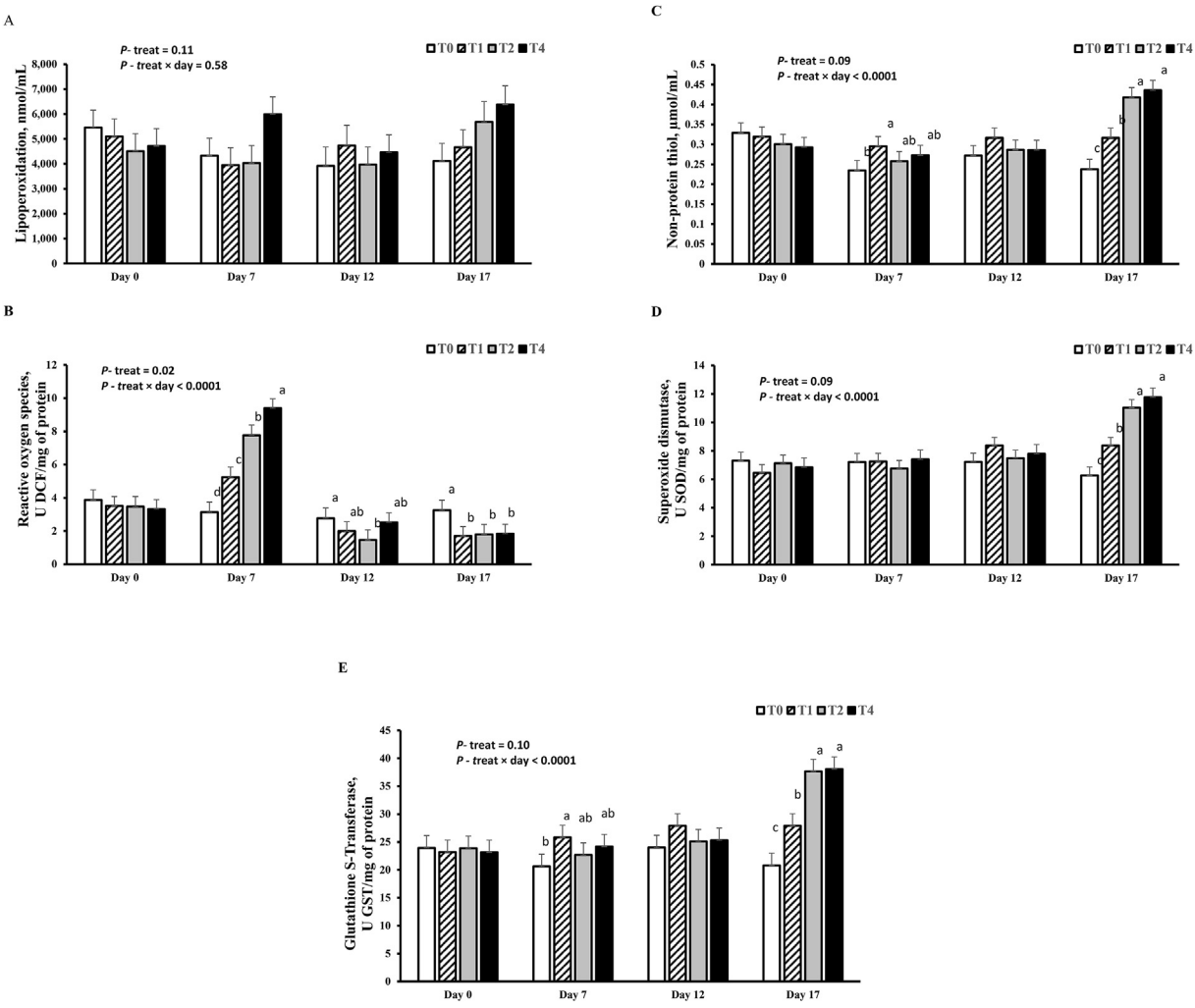

Fig. 2. Serum levels of (A) lipoperoxidation (LPO) (B) reactive oxygen species (ROS) (C) non-protein thiol (NPSH) (D) superoxide dismutase (SOD) and (E) glutathione S-transferase (GST). T0, T1, T2 and T4 represents supplementation of polymethacrylate (Eudragit L-100) nanocapsules loaded with curcumin (N-CU) at 0, 1, 2 and 4 mg/kg concentrate, respectively. DCF = fluorescent dichloro fluorescein. Vertical bars represent the SEM. a–c Different letter differs ($P \leq 0.05$) or tends to differ ($P \leq 0.10$) between treatments each respective day.

increasing glycogen content and inhibiting glucose-6-phosphate activity (Seo et al., 2008). (Javidi et al., 2019) found that curcumin reduced blood glucose levels in streptozotocin-induced diabetes in rats, concluding that the endocrine part of pancreas was restored in curcumin-treated rats, i.e., the positive effects curcumin was associated with protective effects of curcumin on pancreatic tissue.

It is important to note again that this study was intended to supplement lambs that previously were prevented from weaning, a critical period known as feed transition and ambience. The experiment period exceeded the time considered to adapt to the diet; nevertheless, the authors are aware that 17 d is a short time, and this is a limitation of the study; therefore, further studies should be performed during other stages of the production cycle and for longer periods, so as to validate the value of this additive as a commercial formulation for small ruminants.

Another result that merits attention in this study is that the dose of 4 mg/kg was not beneficial for growth and health of lambs in the short term, as it did not stimulate weight gain and also had an inflammatory effect, stimulating leukocyte production; this is not desirable in farm animals, as already mentioned. In addition, increased liver enzymes may suggest liver overload or toxicity of curcumin nanocapsules. A relatively high number of reports suggest that curcumin may cause toxicity under specific conditions (Burgos-Morón et al. (2009)). These authors reflect on the dark side of curcumin in the short- and long-term diet, as well as report several studies that have described undesirable effects of this molecule. With nanoencapsulation, greater absorption of curcumin may have occurred; and this may cause a small degree of toxicity that might give rise to unwanted and unexpected effects.

5. Conclusion

N-CU in powder as supplements in lambs had antioxidant and anti-inflammatory effects (2 mg/kg) and improved growth performance. Regression analysis showed that dietary supplementation of N-CU at 1.89 mg/kg concentrate maximized weight gain. These data suggest that N-CU supplements improve health and performance of pre-weaning lambs. Nanocapsules potentiate the effects of curcumin; they may emerge as new tools in animal nutrition; nevertheless, further studies must be done to validate their long-term safety and efficacy.

Author contributions

H. Marcon and **A.S. Da Silva** contributed to the design and implementation of the research, to the analysis of the results. **A.F. Ourique** and **M. Vedovatto** helped in the elaboration of the project and its execution and financing. **V.L. Molosse**, **L.G. Griss**, **B.G.O. Cecere**, **D.F. Alba**, **K.W. Leal** and **G.M. Galli** participated in the execution of the experiment and collection of samples and data. **C.F. Souza**, **M.D. Baldissera**, **S. Gundel**, and **V.A. Bassotto** did the laboratory analysis. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

We declare that we have no financial and personal relationshipswith other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgment

Thanks to FAPESC (Santa Catarina, Brazil), CNPq (Brazil) and CAPES (Brazil) for their technical support.

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