DOI: 10.5433/1679-0359.2017v38n2p919

Performance and serum chemistry profile of lambs fed on rations with increasing levels of urea

Desempenho e perfil metabólico de cordeiros alimentados com ração contendo níveis crescentes de ureia

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Abstract

Feed intake and efficiency, animal performance, and protein and energy metabolism were studied in lambs that were fed on increasing levels of urea (0.0, 0.5, 1.0, and 1.5% of dry matter [DM]). A completely randomized design was used, with four treatments (urea levels) and six replicates. The lambs were fed *ad libitum* for 56 days until they reached an average body weight of 35 kg. The rations were composed of 34% Tifton 85 hay (*Cynodon* spp.) and 66% concentrate. Crude protein (CP) constituted 17% of the diet on a dry matter basis, and total digestible nutrients (TDN) accounted for 65%, on average. The dry matter intake (DMI) and crude protein intake (CPI) were not influenced by the urea levels in the diet, presenting average values of 1.175 and 0.206 kg animal^{-1} day^{-1}, respectively. The average daily gain (ADG), feed conversion ratio (FCR) and gross feed efficiency (GFE) were also not influenced by urea levels (0.225 kg day^{-1}, 5.33 kg DM kg gain^{-1} and 0.195 kg gain kg DM^{-1}, respectively). Except for urea and glucose concentrations, blood parameters did not change with increasing urea in the diets. The mean values for total protein, albumin, globulin and creatinine in the serum were 7.11 g dL^{-1}, 3.36 g dL^{-1}, 3.75 g dL^{-1} and 0.91 mg dL^{-1}, respectively. Serum urea decreased linearly and serum glucose increased linearly with urea levels in the diet. The addition of 1.5% of urea to the diets did not change feed intake and efficiency or animal performance, and did not cause metabolic disorders in feedlot lambs in the finishing phase.

Key words: Blood. Finishing. Glucose. Nitrogen. Sheep.

Resumo

Avaliou-se o consumo de alimento, o desempenho animal, a eficiência alimentar, e o metabolismo proteico e energético de cordeiros alimentados com rações contendo níveis crescentes de ureia (0,0; 0,5; 1,0; 1,5% da matéria seca – MS). O delineamento foi inteiramente casualizado com quatro tratamentos (níveis de inclusão de ureia) e seis repetições por tratamento. Os cordeiros foram alimentados à vontade por 56 dias até atingirem peso corporal (PC) médio de 35 kg. As rações foram compostas por 34% de feno de Tifton 85 (*Cynodon* spp.) e 66% de concentrado, com 17% de proteína bruta (PB) e 65% de nutrientes digestíveis totais (NDT), em média, com base na matéria seca (MS). O consumo de matéria seca (CMS) e de proteína bruta (CPB) não foram influenciados pelos níveis de inclusão de ureia na ração, apresentando valores médios de 1,175 e 0,206 kg animal^{-1} dia^{-1}. O ganho médio diário (GMD), a

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Received: Apr. 25, 2016 – Approved: Nov. 21, 2016

conversão alimentar (CA) e a eficiência alimentar bruta (EAB) também não foram influenciados pelos níveis de inclusão de ureia e apresentaram valores médios de 0,225 kg dia^{-1}, 5,33 kg de MS kg de $ganho^{-1}$ e 0,195 kg de ganho kg de MS^{-1}. Com exceção da ureia e da glicose, os metabólitos sanguíneos não foram influenciados pelos níveis de inclusão de ureia na ração. Os valores médios para as concentrações séricas de proteínas totais, albumina, globulinas e creatinina foram 7,11 g dL^{-1}, 3,36 g dL^{-1}, 3,75 g dL^{-1} e 0,91 mg dL^{-1}. A ureia sérica apresentou resposta linear decrescente, enquanto a glicose sérica de apresentou resposta linear crescente ao aumento da inclusão de ureia. A inclusão de até 1,5% de ureia na MS em rações isoproteicas não afeta o consumo de alimento, o desempenho e a eficiência alimentar, e não causa prejuízos metabólicos em cordeiros confinados na fase de terminação.
Palavras-chave: Glicose. Nitrogênio. Ovinos. Sangue. Terminação.

Introduction

Appropriate nutrition is important in any production system, constituting a critical point in the process, especially when it involves production in feedlots (GONZAGA NETO et al., 2006). In this system, weight gain should be maximized by providing diets with a high energy content and adequate protein to meet the demand of the animals (HADDAD; HUSEIN, 2004). These diets allow for the early slaughter of lambs, reducing the cost of food and making it an economically viable system.

Protein ingredients have the highest cost per unit in the composition of rations (QUINTÃO et al., 2009). Sources of non-protein nitrogen (NPN), such as urea, may have a lower cost than traditional protein sources (such as soybean meal and cotton) for equivalent quantities of nitrogen (N). The N use efficiency from the NPN sources depends on several factors, including the timing of the release of ammonia, resulting from the hydrolysis of urea, and the presence of energy for microbial protein synthesis in the rumen. In scenarios of high NPN in relation to crude protein (CP) in the diet, and low organic protein fraction (such as amino acids and peptides) that is degradable in the rumen, animal performance can be impaired (PESSOA et al., 2009).

In animal performance studies, it is important to evaluate the intake and digestibility of nutrients, feed conversion ratio and weight gain (PEREIRA et al., 2008). From a nutritional point of view, weight gain is the result of voluntary feed consumption, which determines the quantity of nutrient intake, while digestibility is a secondary factor that is related to the quality of the food (VAN SOEST, 1994). The metabolic challenge imposed by the intensification of animal production has caused an imbalance between the demand for nutrients by the body, the ability to metabolize them and the level of production to be achieved (GONZÁLEZ, 2000). In this context, the characterization of metabolic profiles is useful to evaluate the animal's nutritional status (PEIXOTO; OSORIO, 2007), and enables a more accurate interpretation of the results of production performance in livestock.

The aim of this study was to evaluate the effect of increasing levels of urea in rations on feed and nutrient intake, performance and feed efficiency, and protein and energy metabolism of feedlot lambs in the finishing phase.

Material and Methods

Experimental protocol

The research and procedures conducted on animals were in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (*Colégio Brasileiro de Experimentação Animal – COBEA*) and were approved by the Animal Care and Use Committee (*Comitê de Ética no Uso de Animais – CEUA*) of the Federal University of Paraná (*Universidade Federal do Paraná – UFPR*), Palotina Campus, under the protocol number 08/2012-CEUA issued by the Commission.

The experiment was conducted at the Center of Studies in Small Ruminants (*Centro de Estudos*

em Pequenos Ruminantes – CEPER) of Palotina Campus of UFPR, located in Palotina, Paraná state, Brazil. Twenty-four Dorper crossbred lambs of 2-5 months old, with a mean ± standard error (SE) body weight (BW) of 25.0 ± 0.9 kg, were used. At the beginning of the experiment, the animals were weighed, identified with ear tags and dewormed (Ivermectina® at a dose of 1 mL 50 kg BW^{-1} subcutaneously). Subsequently, the animals were housed in individual, covered pens with a slatted floor. The pens were 1.5 m² and contained a water drinker and individual feeder.

The design was completely randomized, with four treatments and six replications. Treatments corresponded to the inclusion of 0.0, 0.5, 1.0 and 1.5% urea in the ration, based on dry matter (DM). Rations were isoproteic, with 17.03% DM of crude protein (CP) on average (Table 1). The same protein content of rations was maintained while altering the forage:concentrate ratio (F:C), which ranged from 25:75 to 40:60 (Table 1). Rations were composed on average of 34% hay DM Tifton 85 (*Cynodon* spp.) and 66% DM of protein-energy concentrate, and were formulated in accordance with the recommendations of NRC (1985) to meet the requirements for moderate weight gain (200 g day^{-1}). The period of adaptation to the diets was 15 days and the trial period was 56 days.

Table 1. Proportion of ingredients and nutritional composition of rations with increasing levels of urea provided for feedlot lambs in the finishing phase.

Component[I]	Urea (% DM)			
	0.0	0.5	1.0	1.5
Ingredients				
Tifton hay 85 (% DM)	36.0	40.0	34.0	25.0
Concentrate (% DM)	64.0	60.0	66.0	75.0
PCC (% DM)[II]	58.0	33.5	15.0	26.0
Soybean hulls (% DM)	5.0	25.0	49.0	19.0
Ground corn (% DM)	0.0	0.0	0.0	27.5
Mineral premix (% DM)[III]	1.0	1.0	1.0	1.0
Urea (% DM)	0.0	0.5	1.0	1.5
Nutritional composition				
DM (%)	86.70	87.90	89.50	88.52
CP (% DM)	16.85	17.01	17.17	17.07
EE (% DM)	2.29	2.24	2.24	2.63
NDF (% DM)	28.55	44.75	56.63	32.71
Ca (% DM)	0.97	0.79	0.69	0.64
P (% DM)	0.46	0.35	0.27	0.36
Ca:P	2.10	2.26	2.56	1.88
TDN (% DM)	65.03	63.00	63.00	68.80
ME (Mcal kg DM^{-1})	2.36	2.27	2.27	2.48

[I] DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; Ca: calcium; P: phosphorus; Ca:P: calcium:phosphorus ratio; TDN: total digestible nutrients; ME: metabolizable energy

[II] PCC – pelletized commercial concentrate: 13% moisture; 18% CP; 2.5% EE; 9% crude fiber (CF); 10% ash; 0.15% Ca; 0.06% P; 0.05% Na; 100 ppm antioxidant butylated hydroxytoluene (BHT); 20 ppm Co; 45 ppm Cu; 55 ppm Fe; 10 ppm I; 50 ppm Mn; 0.3 ppm Se; 1000 IU Vit A; 5,800 IU Vit D_3; 600 IU Vit E

[III] 1.2% Mg; 13.3% Na; 1% S; 6.5% P; 16.2% Ca; 2250 ppm Mn; 86 ppm Cu; 1400 ppm Fe; 200 ppm Co; 23 ppm Se; 4500 ppm Zn; 177 ppm I; 100,000 IU Vit A; 65,000 IU Vit D_3; 60 IU Vit E.

The diets were supplied as total mixed ration (TMR) and split into two daily meals (8:00 and 14:00). Hay was cut into particles of approximately 3 cm in length to improve utilization by the animals and to reduce waste in the feeder. The animals were fed *ad libitum* in the adaptation period and during the experiment, keeping the orts at 10% of the amount of feed provided. Adjustments to the amount of ration supplied were performed every five days based on the amount of orts.

The trial period was set by the time required for the animals' final BW to reach 35 kg, on average, which corresponds to the average BW at slaughter for lambs in the region (30-40 kg). In order to evaluate DM and nutrient intake, performance, and protein and energy metabolism profiles in lambs at the beginning and at the end of the finishing phase, the experimental period was divided into two periods of 28 days (Initial Period: 0-28 days; Final Period: 29-56 days).

Measurements

The orts of each animal were collected, weighed and stored in plastic bags daily. At the end of each week, composite samples were formed from the orts collected over the previous seven days. Subsamples of composite samples from each week were collected and stored in a freezer at a temperature of –4°C. The sub-samples were thawed at the time of the chemical analysis, and representative composite samples of the initial period and the final period were formed and sent for analysis.

Orts samples were dehydrated in a forced ventilation oven at 65°C for 72 h, and were subsequently processed in a Wiley knife mill with a 1 mm mesh sieve. The chemical analysis included the quantification of DM, CP, ether extract (EE) and ash according to the methods proposed by the AOAC (1990), and neutral detergent fiber (NDF) according to the methodology described by Van Soest et al. (1991). From these nutrients, the non-fibrous carbohydrate (NFC) was calculated according to Hall (2000) and total digestible nutrients (TDN) were calculated according to Weiss et al. (1992). Drying, sample processing and chemical analysis were performed at the Laboratory of Animal Nutrition (*Laboratório de Nutrição Animal - LANA*) of the UFPR, Palotina Campus.

The daily intake of dry matter (DMI), crude protein (CPI), ether extract (EEI), neutral detergent fiber (NDFI) and total digestible nutrients (TDNI) was calculated for each animal as the difference between the amount of DM and nutrients in the feed provided and in the orts. DMI and nutrient intake were expressed in kg day^{-1} and % BW day^{-1}.

Performance of the lambs was evaluated using weight measurements in the initial period and the final period. Weights were measured early in the morning after fasting for 12 h. The total weight gain (WG, kg) was calculated as the difference between initial and final BW of lambs in each finishing phase; the average daily gain (ADG, kg day^{-1}) was obtained by dividing the WG by the number of days of each period of finishing phase. Feed conversion (FCR, kg DM kg $gain^{-1}$) was calculated by the ratio between DMI and ADG; gross feed efficiency (GFE, kg gain kg DM^{-1}) corresponded to the inverse of the FCR and was calculated as the ratio of ADG to DMI (SANTANA et al., 2012). FCR and GFE were also calculated for each period of finishing phase.

After the lambs were weighed, blood samples were collected to evaluate metabolic profiles. To assess the dynamics of blood metabolites after feeding, blood samples were collected at 0, 2, 4, 8 and 12 h postprandial. The protocol was: (1) provide the first meal for the lambs after weighing; (2) collect the orts 1 h after the first meal; (3) after cleaning the feeders (time 0), sample the blood at the aforementioned times.

To facilitate the serial blood sampling and reduce the stress to the animals, samples were collected through a catheter applied and fixed on the lambs' necks one day before the weighing, according to the method described by Krehbiel et al. (1995). The catheter was inserted into the jugular vein in the caudal-ventral direction, and after confirming

its correct position, the needle was removed and a PRN adapter was coupled to the catheter. A saline solution containing heparin (approximately 3 ml) was applied in the catheter after each sampling to prevent blood clotting and avoid its obstruction.

Blood samples were collected with a 10 mL syringe, which was the volume of sample collected at each sampling time. After collection, samples were centrifuged for 10 minutes at 3,600 RPM to obtain the serum. After centrifugation, the serum was stored in Eppendorf microtubes (1.5 ml) and was frozen. Serum samples remained frozen until the time of biochemical analyses, which were conducted in the Laboratory of Clinical Analysis of Veterinary Hospital (*Laboratório de Análises Clínicas do Hospital Veterinário*) of the UFPR, Palotina Campus. Using Labtest® biochemical kits and the semi-automatic biochemical analyzer Quicklab II – Drake, the concentrations in the serum of total protein (colorimetric method of biuret; Ref. No. 99), albumin (colorimetric method of bromocresol green; Ref. No. 19), creatinine (colorimetric method of Jaffé, Ref. No. 96), urea (colorimetric method of urease; Ref. No. 27) and glucose (kinetic method of glucose oxidase; Ref. No. 134) were determined. The serum globulin was calculated as the difference between serum concentrations of total protein and albumin.

Statistical analysis

Data on the DM and nutrient intake and the lamb's performance were analyzed in a split plot model, in which the plot was characterized by the urea levels in the ration and the subplots corresponded to periods of the finishing phase (initial and final period). The isolated effects of urea levels and period of finishing, as well as the interaction between the two factors, were tested in an analysis of variance (PROC GLM) at 5% significance level. When the isolated effect of urea levels was significant, regression analysis (PROC REG) until the second order (quadratic) was performed. When the isolated effect of period of finishing was significant, means were compared using F test. When the interaction between the two factors was significant, regression analysis for levels of urea was carried out within each period of finishing.

Metabolic profile data were analyzed in a split-split plot model, in which the plot was characterized by the urea levels in the ration, the subplots were the periods of the finishing phase (initial and final period) and sub-subplots were the times of blood sampling (0, 2, 4, 8 and 12 h postprandial). The isolated effects of urea levels, period of finishing and time of blood sampling, as well as the interactions among the three factors, were tested in an analysis of variance (PROC GLM) at 5% level of significance. When the isolated effect for urea levels and for period of finishing were significant, the same analysis described above for DM and nutrient intake and performance data were performed. When there was an isolated effect of time of blood sampling, a regression analysis (PROC REG) of the second order (quadratic) was performed. In the case of a significant interaction among the three factors, developments were carried out considering the hierarchy of factors (plot, subplots and sub-subplots), and the same analysis that was applied to isolated effects was performed (QUINN; KEOUGH, 2004). Analyses were performed using the Statistical Analysis System (SAS) version 9.0.

Results and Discussion

The DMI and the CPI were not affected ($p > 0.05$) by urea levels in the ration or the periods of the finishing phase (Table 2), with average values of 1.175 and 0.206 kg animal^{-1} day^{-1}, respectively. The average values of DMI for all feeds and in both finishing periods met the recommendation of the NRC (1985) for moderate to fast growth rates (1.0 to 1.3 kg animal^{-1} day^{-1}). The DMI can be reduced when the true protein replaced by NPN exceeds 30% of the total diet N (RINDSIG, 1977). In this study, the maximum level of urea was 1.5% DM of the feed, which represented 23.3% of total dietary N. Under these conditions, the DMI of lambs was not reduced.

Table 2. Mean and standard error of the mean (SEM) for dry matter and nutrient intake by feedlot lambs fed on rations with increasing levels of urea at the beginning and at the end of the finishing phase.

Variables[I]	Urea (% DM)				Mean (SEM)	Regression[II]
	0.0	0.5	1.0	1.5		
Initial period (0-28 days)						
DMI (kg day^{-1})	1.087	1.175	1.168	1.132	1.139 (0.033)	NS
CPI (kg day^{-1})	0.191	0.205	0.202	0.198	0.199 (0.006)	NS
EEI (kg day^{-1})	0.028	0.027	0.028	0.032	0.029 (0.001)	NS
NFDI (kg day^{-1})	0.245	0.451	0.655	0.353	0.426 (0.035)	NS
TDNI (kg day^{-1})	0.753	0.760	0.742	0.777	0.758 (0.091)	NS
DMI_{BW} (% BW day^{-1})	4.13	3.91	4.07	4.02	4.04 (0.058) a	NS
CPI_{BW} (% BW day^{-1})	0.72	0.69	0.72	0.70	0.70 (0.009) a	NS
EEI_{BW} (% BW day^{-1})	0.10	0.10	0.10	0.10	0.11 (0.001) a	NS
$NFDI_{BW}$ (% BW day^{-1})	1.58	1.56	1.56	1.41	1.53 (0.111)	NS
$TDNI_{BW}$ (% BW day^{-1})	2.72	2.53	2.63	2.63	2.62 (0.035) a	NS
Final period (29-56 days)						
DMI (kg day^{-1})	1.199	1.213	1.240	1.189	1.210 (0.031)	NS
CPI (kg day^{-1})	0.211	0.213	0.217	0.206	0.212 (0.006)	NS
EEI (kg day^{-1})	0.030	0.029	0.029	0.033	0.030 (0.001)	NS
NFDI (kg day^{-1})	0.228	0.483	0.676	0.336	0.428 (0.039)	NS
TDNI (kg day^{-1})	0.799	0.778	0.831	0.839	0.815 (0.023)	NS
DMI_{BW} (% BW day^{-1})	3.65	3.62	3.65	3.50	3.61 (0.770) b	NS
CPI_{BW} (% BW day^{-1})	0.64	0.64	0.64	0.61	0.63 (0.014) b	NS
EEI_{BW} (% BW day^{-1})	0.09	0.09	0.09	0.10	0.09 (0.002) b	NS
$NFDI_{BW}$ (% BW day^{-1})[III]	0.70	1.44	1.99	0.99	1.27 (0.112)	***
$TDNI_{BW}$ (% BW day^{-1})	2.43	2.43	2.56	2.48	2.47 (0.047) b	NS

[I] DMI: dry matter intake; CPI: crude protein intake; EEI: ether extract intake; NDFI: neutral detergent fiber intake; TDNI: total digestible nutrients intake; DMI_{BW}: DMI relative to body weight (BW); CPI_{BW}: CPI relative to BW; EEI_{BW}: EEI relative to BW; $NFDI_{BW}$: NFDI relative to BW; $TDNI_{BW}$: TDNI relative to BW

[II] NS: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$

[III] $NDFI_{BW} = 0.6334 + 2.8921U - 1.7382U^2$ ($R^2 = 0.9000$)

Means for the same variable followed by different letters in the same column differ according to F test ($p < 0.05$).

The NDF intake showed a quadratic relationship ($p < 0.05$) with urea (U) levels in the rations (NDF = $0.2126 + 0.9260U - 0.5475U^2$; $R^2 = 0.8806$), regardless of the period of finishing. The NDF intake is related to the NDF of rations, which were 28.55, 44.75, 56.63 and 32.71% DM for rations with 0.0, 0.5, 1.0 and 1.5% DM of urea, respectively (Table 1). The maximum value of 0.665 kg DM animal^{-1} day^{-1} for NDF was observed in the ration with 1.0% DM of urea, which showed higher NDF among the evaluated diets.

To make isoproteic rations, it was not possible to keep the NDF and the F:C constant in the evaluated diets (Table 1). Among the concentrate ingredients used in the formulations, soybean hulls were added at levels of 5-49% of the feed DM, with a maximum value of inclusion in the ration with 1.0% DM urea. As soybean hulls have approximately 60% DM of NDF, they were initially considered as an option to replace forage in the diet (RESTLE et al., 2004). However, due to the high digestibility of NDF, the soybean hulls provide high production of short chain fatty acids, which has positive effects on animal performance (BACH et al., 1999). In addition to the high digestibility of NDF, soybean hulls have high pectin content. According to the NRC (2001), pectin represents 62.4% of the fraction of non-fiber carbohydrates (NFC) of soybean hulls, and its cell wall has little lignin, which maximizes the

use of structural carbohydrates by microorganisms. Pectin differs from starch by the axial position of the carbon four bond, so that it is not attacked by digestive enzymes but it is susceptible to microbial action (MORGADO et al., 2009). In addition to not producing lactic acid during fermentation, the structure of pectin (monosaccharide galacturonic acid) promotes an efficient buffering of the rumen through its cation exchange capacity and links with metal ions (VAN SOEST et al., 1991). Thus, it can be inferred that the use of soybean hulls contributed to the avoidance of a reduction in DMI with the inclusion of urea in feed, which may be related to the timing of degradation of NPN and carbohydrate by rumen microorganisms. Other factors related to the soybean hulls that may explain the constant DMI in relation to the increase in NDF are reduced particle size and high hydration capacity of this ingredient. These features help to increase the passage rate of NDF through the gastrointestinal tract, resulting in no change to DMI (IPHARRAGUERRE; CLARK, 2003).

The DMI, CPI, EEI and TDNI in relation to BW were not affected ($p > 0.05$) by urea levels in the diet, but were higher ($p < 0.05$) in the initial period of the finishing phase (Table 2). The decrease in DMI and nutrient intake in relation to BW was expected, because as the lambs grow, their metabolic rate decreases, causing a decrease (proportional to BW) in the requirement of DM intake and nutrients to meet the demand for maintenance and growth.

There was quadratic effect of urea levels in the ration ($p < 0.05$) on the NDF in relation to the BW ($NDFI_{BW}$) at the final period of the finishing phase (Table 2). As with the NDF, differences in NDF and the F:C of the rations explain the quadratic response of $NDFI_{BW}$ for urea levels at the final period of the finishing phase.

The performance of the lambs and feed efficiency traits were not affected ($p > 0.05$) by urea levels in the ration (Table 3). Other studies also showed no effect of increasing levels of urea in the diet on the ADG and FCR in lambs (OKAMOTO et al., 2008; ZIGUER et al., 2012). Mean values for ADG, FCR and GFE were 0.225 kg day^{-1}, 5.33 kg DM kg $gain^{-1}$ and 0.195 kg gain kg DM^{-1}, respectively. The ADG was within the range indicated by the NRC (1985) for lambs showing moderate to fast growth rates, implying that the ADG results obtained in this study were satisfactory.

Except for WG and FCR, traits showed higher values at the final period of finishing (Table 3) when compared with the same traits at the initial period. The highest ADG and GFE values at the end of the finishing phase indicated a better use of urea by lambs during this period. The hydrolysis rate of urea in the rumen decreases over time (OWENS; ZINN, 1993), meaning that there may be a decrease in animal performance for about one month, then a return to normal growth rates after this adaptation period.

With the exception of serum urea and glucose, blood metabolites were not affected ($p > 0.05$) by urea levels in the ration. The average values for serum total protein, albumin, globulins and creatinine were 7.11 g dL^{-1}, 3.36 g dL^{-1}, 3.75 g dL^{-1} and 0.91 mg dL^{-1}, respectively.

Table 3. Mean and standard error of the mean (SEM) for performance and feed efficiency traits of feedlot lambs fed on rations with increasing levels of urea at the beginning and at the end of the finishing phase.

Variables[I]	Urea (% DM)				Mean (SEM)	Regression[II]
	0.0	0.5	1.0	1.5		
Initial period (0-28 days)						
Final BW (kg)	30.70	30.10	31.50	31.00	30.82 (1.005) b	NS
WG (kg)	4.88	5.97	5.53	6.52	5.72 (0.253)	NS
ADG (kg day^{-1})	0.188	0.199	0.185	0.231	0.201 (0.010) b	NS
FCR (kg DM kg gain^{-1})	5.40	5.64	6.39	5.07	5.64 (0.238)	NS
GFE (kg gain kg DM^{-1})	0.174	0.188	0.157	0.204	0.181 (0.008) b	NS
Final period (29-56 days)						
Final BW (kg)	36.10	36.50	36.50	36.90	36.50 (1.005) a	NS
WG (kg)	5.43	5.52	5.10	5.90	5.49 (0.244)	NS
ADG (kg day^{-1})	0.247	0.251	0.232	0.268	0.249 (0.022) a	NS
FCR (kg DM kg gain^{-1})	4.93	5.01	5.47	4.64	5.01 (0.203)	NS
GFE (kg gain kg DM^{-1})	0.208	0.212	0.188	0.223	0.208 (0.009) a	NS

[I] BW: body weight; WG: weight gain; ADG: average daily gain; FCR: feed conversion ratio; GFE: gross feed efficiency
[II] NS: not significant; * p <0.05; ** p <0.01; *** p <0.0001
Means for the same variable followed by different letters in the same column differ according to F test ($p < 0.05$).

Table 4. Mean and standard error of the mean (SEM) for serum concentrations of blood metabolites determined at 0-12 hours after feeding in feedlot lambs fed on rations with increasing levels of urea at the beginning and at the end of the finishing phase.

Variables	Postprandial time (hours)					Mean (SEM)
	0	2	4	8	12	
Initial period (0-28 days)						
Total protein (g dL^{-1})[I]	8.14	8.70 a	6.56 b	5.43	7.31 a	7.23 (0.16)
Albumin (g dL^{-1})[II]	3.96 a	4.08 a	3.65	3.24	3.03 b	3.59 (0.07) a
Globulins (g dL^{-1})[III]	4.18 b	4.63	2.91 b	2.20	4.28 a	3.64 (0.14)
Creatinine (mg dL^{-1})	0.86 b	0.78 b	0.94	0.83	0.80 b	0.84 (0.01) b
Urea (mg dL^{-1})	54.83	79.04	58.96	49.04	54.38	59.25 (1.50) b
Glucose (mg dL^{-1})	82.90	91.92	95.88	90.67	80.19	88.31 (1.33) a
Final period (29-56 days)						
Total protein (g dL^{-1})[I]	7.94	7.27 b	7.47 a	5.62	6.47 b	6.98 (0.12)
Albumin (g dL^{-1})[II]	2.57 b	2.70 b	3.50	3.19	3.66 a	3.13 (0.05) b
Globulins (g dL^{-1})[III]	5.37 a	4.56	3.97 a	2.42	2.80 b	3.85 (0.13)
Creatinine (mg dL^{-1})[IV]	1.17 a	0.96 a	0.98	0.94	0.85 a	0.98 (0.02) a
Urea (mg dL^{-1})	70.52	73.57	56.96	56.02	56.63	62.74 (1.09) a
Glucose (mg dL^{-1})	83.82	61.00	70.50	73.76	74.93	72.71 (1.51) b

[I] $TP_{INITIAL} = 8.832 – 0.741U + 0.049U^2$ ($R^2 = 0.6570$)
$TP_{FINAL} = 8.091 – 0.403U + 0.021U^2$ ($R^2 = 0.7450$)
[II] $ALB_{INITIAL} = 4.0594 – 0.0901U$ ($R^2 = 0.9199$)
$ALB_{FINAL} = 2.5857 + 0.1606U – 0.0065U^2$ ($R^2 = 0.7291$)
[III] $GLOB_{INITIAL} = 4.759 – 0.642U + 0.048U^2$ ($R^2 = 0.6290$)
$GLOB_{FINAL} = 5.505 – 0.563U + 0.0270U^2$ ($R^2 = 0.9620$)
[IV] $CREAT_{FINAL} = 1.0867 – 0.0206U$ ($R^2 = 0.7073$)
Means for the same variable followed by different letters in the same column differ according to F test ($p < 0.05$).

Serum urea decreased linearly ($p < 0.05$) with the inclusion of urea in the ration (Urea = 64.37 – 4.21U; $R^2 = 0.6878$). Whereas microorganisms correctly use ammonia (NH_3) when there is adequate energy intake, increasing the proportion of carbohydrate sources with high degradation rates in the feed with higher levels of urea (1.0-1.5% DM; Table 1) may result in a better use of NH_3 for microbial protein synthesis. This may explain the decrease in serum urea with increasing urea levels in the ration. The greater availability of energy could be due to the increased proportion of soybean hulls in diets with 0.5 and 1.0% DM of urea, and the inclusion of maize in the diet with 1.5% DM of urea. The highest level of serum urea was found in lambs fed rations without the addition of urea, which may be attributed to the use of low fermentation potential sources in the commercial concentrate.

The serum glucose increased linearly ($p < 0.05$) with the inclusion of urea in the ration (Glucose = 77.57 + 4.42U; $R^2 = 0.9228$). A similar result was reported by Noro et al. (2012) when evaluating the effects of three levels of urea in feed (0.00, 0.55 and 1.28 g N kg of metabolic weight^{-1}) on the energy metabolism of lambs. The authors attributed the higher glucose levels in lambs fed on a diet with high NPN to high phosphoenolpyruvate carboxykinase (PEPCK) enzyme activity in the liver, which plays an important role in hepatic gluconeogenesis process. It is likely that in the present study there was an increase in the gluconeogenic capacity of the liver with increasing inclusion of urea in the ration, resulting in increased blood glucose in lambs.

Serum total protein showed a quadratic response ($p < 0.05$) at the sampling times at the beginning and at the end of the finishing phase (Table 4). Serum concentrations of this metabolite remained within the reference range for this species in the sampling times (6.0-7.9 mg dL^{-1}) (KANEKO et al., 2008). Variations in serum total protein among sampling times, and between the periods of finishing, may be related to changes that occur in the animal as it modifies the composition of gain as the finishing phase progresses. It is likely that in the first period, there was a higher protein demand for muscle deposition. Therefore, urea was efficient in the synthesis of microbial protein, which ensured adequate levels of amino acids. This did not cause metabolic disorders because the protein is mainly synthesized in the liver in order to ensure its contribution to other tissues.

Serum albumin decreased linearly ($p < 0.05$) at sampling times in the initial period of finishing, and exhibited a quadratic effect at the sampling times in the final period of finishing (Table 4). The serum albumin was maintained at values close to the reference for this species (2.4-3.0 g dL^{-1}) (KANEKO et al., 2008), with higher values at the sampling times 0 and 2 h in the initial period of finishing (3.96 and 4.08 g dL^{-1}, respectively; Table 4).

Serum globulins showed a quadratic response ($p < 0.05$) with the sampling times in the two periods of the finishing phase (Table 4). There was no effect of urea levels on this metabolite, implying that urea did not suppress the immune system. The variation in serum globulins during fasting may be related to the variation in water intake during this period (RUSSELL; ROUSSEL, 2007).

Serum creatinine decreased linearly ($p < 0.05$) over the sampling times in the final period of finishing (Table 4). The average values for serum creatinine at the beginning and end of the finishing phase remained slightly below the reference range for sheep (1.2-1.9 mg dL^{-1}) (KANEKO et al., 2008), which is justified by the lower muscle development of lambs compared to adult animals. Creatinine is formed during the activity of skeletal muscle and is used as an index for the glomerular filtration rate, and as with urea, a reduction in glomerular filtration increases the serum creatinine (MEYER et al., 1995). Therefore, the higher serum creatinine at the beginning of the sampling period may be related to the metabolism of nutrients in the muscle and the excretion of metabolites from muscle after feeding.

Conclusion

The inclusion of up to 1.5% DM of urea in isoproteic diets did not significantly affect the DM intake, performance or feed efficiency of lambs during the finishing phase, which justifies its use in order to reduce production costs. Serum concentrations of metabolites related to protein and energy metabolism remained within acceptable ranges for sheep, suggesting that the inclusion of up to 1.5% DM of urea in rations does not cause metabolic damage to young sheep.

Acknowledgments

The authors acknowledge the contribution of Luciana Helena Kowalski during experimental preparation and data collection and Tiago Machado dos Santos in the analysis procedures in LANA of UFPR, Palotina Campus.

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