Received: 7 December 2017 | Revised: 30 May 2018 | Accepted: 31 May 2018

DOI: 10.1111/jpn.12945

ORIGINAL ARTICLE

WILEY Journal of Animal Physiology and Animal Nutrition

Effects of pearl millet silage ammoniation with urea on carcass and meat quality of lambs

Gleidson G. P. de Carvalho[1] | Poliane Meire D. de Freitas[1] | Edson M. Santos[2] | Gherman G. L. Araújo[3] | Juliana S. de Oliveira[2] | Thomaz Cyro G. de C. Rodrigues[1] | Maria Leonor G. M. L. de Araujo[1] | Dallyson Yehudi C. de Assis[1] | Roberto de O. Roça[4] | Nara Laiane C. de Delbem[5] | Renata L. Lomele[5] | Laudi C. Leite[6] | Carlindo S. Rodrigues[7]

[1]Department of Animal Science, School of Veterinary Medicine and Animal Science, Federal University of Bahia, Salvador, Brazil

[2]Federal University of Paraíba, Areia, Brazil

[3]*Embrapa Semiárido*, Petrolina, Brazil

[4]Faculty of Agronomic Sciences (FCA), UNESP, Botucatu, Brazil

[5]Graduate Student in Animal Science, FMVZ, UNESP, Botucatu, Brazil

[6]Federal University of Recôncavo da Bahia, Cruz das Almas, Brazil

[7]Instituto Federal de Educação, Ciência e Tecnologia Baiano (IFBaiano), Uruçuca, Brazil

Correspondence
G.G.P. de Carvalho, Department of Animal Science, School of Veterinary Medicine and Animal Science, Federal University of Bahia, Salvador, Brazil.
Email: gleidsongiordano@yahoo.com.br

Abstract

This study aimed to evaluate the effect of pearl millet silage ammoniated with urea on lamb carcass characteristics and meat quality. Thirty-two noncastrated crossbred lambs at 4–5 months of age, with an average initial body weight of 17.39 ± 2.16 kg, were distributed into four treatments in a randomized block experimental design with eight animals per treatment. Experimental diets were composed of pearl millet silage ammoniated with four levels of urea (0%, 2%, 4% and 6%, on dry matter basis (DM). Carcass conformation and fatness decreased linearly ($p < 0.05$). Except for the fat content, the proximate composition was not influenced ($p < 0.05$) by the diets. Meat lightness and yellowness increased linearly ($p < 0.05$). There was no effect of diets ($p > 0.05$) on cooking losses or shear force. The levels of oleic and erucic fatty acid levels in the meat decreased linearly ($p < 0.05$), whereas linoleic and eicosadienoic acids, polyunsaturated fatty acids, PUFA:SFA ratio and ω6 contents increased ($p < 0.05$). Pearl millet silage ammoniated with urea allows for the production of good-quality lamb meat, with greater concentrations of polyunsaturated fatty acids. However, lambs fed diets with pearl millet ammoniated with up to 6% lead to a reduction of carcass characteristics without significantly affecting loin-eye area possibly associated with low palatability of the additive used. The lower acceptance of the silages with higher levels of urea is due to the ammonia retention in the material is attributed to the ammonization process. Thus, in spite of the benefits on lamb meat quality, it is suggested that the use of this additive in the ammonization of tropical forages be carried out with care, in limits of up to 6%.

KEYWORDS

commercial cuts, fatty acids, proximate composition, sheep, yield

1 | INTRODUCTION

The difficulty obtaining a regular supply of good-quality feedstuffs all year long is one of the major obstacles faced by sheep farmers in semi-arid regions of the world. Therefore, forage preservation is an alternative for animals reared in regions with long periods of drought, as it can prevent weight losses and declines in production performance (Santos et al., 2015).

Aurousseau et al. (2007) emphasized the need to discover new types of forage with potential to generate good-quality lamb meat. In this regard, feedstuffs to compose diets for feedlot lambs have been extensively researched in the last few years (Flaten, Bakken, & Randby, 2015; Przemyslaw et al., 2015; Silva et al., 2016; Soltaninezhad, Dayani, Khezri, & Tahmasbi, 2016), and these studies have focused mainly on the use of forage preserved as silage and hay.

Feed costs limit intensive animal rearing in many countries, including Brazil (van Cleef et al., 2016). According to these authors, alternative feed sources are adopted to reduce production costs without affecting the generation of animal products like milk, meat and wool. However, the scientific literature has shown that the diet can influence the fatty acid profile, antioxidant properties, rate of protein synthesis, colour, flavour, texture and other quality-related properties of meat (Kim, Stuart, Rosenvold, & Maclennan, 2013; Wood et al., 2004; Young, Cruickshank, MacLean, & Muir, 1994). It is important to evaluate these effects, as the main goal of animal husbandry is the generation of high-quality meat to meet the demands and expectations of the consumer market (Jiang et al., 2015).

Pearl millet (*Pennisetum glaucum* L.) is one of the alternative species with high forage quality and considerable resistance to water scarcity, which makes it the ideal forage for regions with rainfall fluctuations and probability of water stress (Bostid, 1996; Jahanzad et al., 2015). As stated by Bostid (Board on Science and Technology for International Development) (1996), this forage species is well-adapted to low-fertility soils and has the potential to produce acceptable amounts of biomass in conditions under which sorghum (*Sorghum bicolor L.*) or corn would not develop satisfactorily. For these reasons, the use of pearl millet substituting corn as the roughage source is an excellent option in ruminant diets, given both its resistance to water stress and its nutritional value similar to that of corn.

In a basic study evaluating only the quality of pearl millet silages without animals, Pinho et al. (2013) indicated pearl millet as a promising forage alternative for semi-arid regions. Despite this, for recommendations to me made about the use of this plant, tests with animals are necessary to measure its effect on the production and quality of meat, among other parameters.

The main objective of silage making, according to Wilkinson and Davies (2013), is to maintain the original quality of the preserved crop as much as possible. For this purpose, additives have been used for several decades to direct the fermentation process towards the production of lactic acid as the main fermentation product.

Seppälä, Heikkilä, Mäki, and Rinne (2016) stressed that the purpose of using additives during ensiling is to inhibit the growth of undesirable micro-organisms so as to prevent spoilage of the feed, thereby minimizing energy and nutrient losses. The use of chemical additives in the ensiling of tropical forages has been tested for over two decades (Felix & Funso, 1994; Mahouachi, Haddad, Kayouli, Théwis, & Beckers, 2003; Miron, Kabala, Tockb, & Ben-Ghedalia, 1995), and the improvement and efficiency of production of preserved roughages (Nkosi et al., 2016; Roth et al., 2016) have been gradually contributing to animal production.

Romão et al. (2013), Chizzotti et al. (2015) and Martins et al. (2015) investigated and described the use of urea and calcium oxide as alkalinizing chemical additives in the ensiling of sugarcane. According to them, these additives have the potential to improve the nutritional value of the forage, reducing its fibre content and increasing its digestibility, because addition of alkaline agents is related to the action of alkaline hydrolysis. In this context, feeding lambs with pearl millet silage treated with urea may be feasible, and it can be used as another roughage alternative for feeding lambs in feedlots especially in tropical regions.

Given the above, it was hypothesized that pearl millet silages treated with urea could increase carcass yield compared to lambs fed silages without additive. So, it is expected that animals will be slaughtered at an earlier stage, because they will consume silages with higher nutritional value. So, diets will allow a better productive performance, and consequently meat quality since it will be required a shorter time of animals in feedlot.

Therefore, this study aimed to evaluate the effect of pearl millet silage ammoniated with urea on carcass characteristics and meat quality of feedlot lambs.

2 | MATERIALS AND METHODS

The experiment was conducted in compliance with the recommendations of the Brazilian Guide for Animal Experimentation. All procedures were approved by the Ethics Committee in Animal Experimentation (Permit Number: 05-2016).

2.1 | Location, experimental design and diets

The experiment was conducted on the Experimental Caatinga Biome Field, at the Metabolism Unit of the Brazilian Agricultural Research Company (Embrapa Semiarid), located in Petrolina–PE, Brazil, between October and December 2013.

Thirty-two vaccinated and dewormed uncastrated, mixed-breed male lambs at 4–5 months of age and with an initial average body weight of 17.39 ± 2.16 kg were used in this experiment. Lambs were housed individually in covered stalls measuring 1.0 × 1.2 m^2, with concrete floor provided with drinkers ad feed troughs, which were available (ad libitum) during the entire experimental period.

The experiment lasted 62 days, the first 10 of which were used for the adaptation of animals to diets and facilities. Lambs were distributed into four treatments in a completely randomized block design with eight replicates in which their weight was adopted as the criterion for allocation into the blocks. Experimental diets were composed of pearl millet silage ammoniated with four levels of urea (0, 20, 40 and 60 g/kg, on a dry matter basis) as the roughage source plus a concentrate consisting of ground corn, soybean and a mineral mix, with different roughage:concentrate ratios (Table 1).

TABLE 1 Composition of ingredients and chemical composition of experimental diets

Ingredient (g/kg DM)	Urea level (g/kg DM)			
	0	20	40	60
Ground corn	190.8	187.4	212.8	182.8
Soybean meal	38.3	35.5	18	40.5
Mineral supplement[a]	17.3	17	19	16.7
Urea	3.5	0	0	0
Pearl millet silage	750	760	750	760
Total	1000	1000	1000	1000
Chemical composition				
Dry matter	382.5	375.5	401.4	371
Organic matter[b]	914.3	908.8	903.3	906.6
Mineral matter[b]	82.1	91.1	96.5	93.5
Crude protein[b]	152.2	158.1	150.1	155.5
Ether extract[b]	25.1	26	25.7	28.4
NDIP[c](g/kg CP)	176.3	201	256.1	245.6
ADIP[d](g/kg CP)	78.4	87.8	109.8	127.9
Neutral detergent fibre ap[e]	445.1	483.2	498.6	528.5
Acid detergent fibre[b]	280	287.8	316.4	346.9
Lignin[b]	32.3	41.5	49.7	62.5
Cellulose[b]	247.7	246.3	266.7	284.4
Hemicellulose[b]	165.4	188.3	184.3	181.1
Nonfibrous carbohydrates[e]	293	242.6	240.2	185.3
Total digestible nutrients[f]	569.2	588.5	596.2	587
pH of the silages	3.7	4.9	6.8	8.7

Notes. [a]Provides per 1,000 mg (minimum): calcium—141.48 g; phosphorus—43 g; sodium—214.50 g; sulphur—16 g; copper—700 mg; cobalt—50 mg; iron—2,700 mg; iodine—50 mg; manganese—1,500 mg; selenium—25 mg; zinc—1,800 mg; chlorine—330 g; fluorine—431 mg. [b]Expressed as a g/kg of DM. [c]NDIP = neutral detergent insoluble protein, [d]ADIP = acid detergent insoluble protein. [e]ap = corrected for ash and protein. [f]Total digestible nutrients estimated by equations of Detmann, Pina et al. (2006); Detmann, Valadares Filho, Pina et al. (2006); Detmann, Valadares Filho, Henriques et al. (2006); Detmann et al. (2007). Analyses performed at the Forage Crops Laboratory at UESB.

2.2 | Experimental diets

The pearl millet (*Pennisetum glaucum* [L.] R. Br.) cultivar used in this study was ADR500, harvested at the chronological age of 72 days, when its grains were in the pasty/doughy stage. To estimate the dry matter (DM) content in the pearl millet, whole plants were collected 4 days before total collection. These were predried in an oven at −55°C for 72 hr, as described by AOAC (1990).

Whole plants were collected mechanically and chopped in a forage chopper coupled to the tractor to particles of approximately 3 cm. Urea was added to the chopped material on the shed floor at the respective treatment levels of 0, 20, 40 and 60 g/kg (on dry matter basis), at a compaction rate of 600 kg of green grass per cubic metre. So, regardless of density, the additive was added to the material without dilution in water because pearl millet had over 70% moisture, which is sufficient to promote ammoniation.

Half plastic drums with 200-L capacity were used as experimental silos. After the pearl millet was weighed and homogenized with the urea levels, the material was placed in the silos, compacted by trampling, sealed with plastic lids and clamps, and finally stored in a covered shed until they were provided to the animals.

Experimental diets were formulated to be isonitrogenous (15% crude protein), following recommendations of the National Research Council (NRC, 2007), for an average daily gain of 200 g. Animals received the feed twice daily, at 08 and 16 hr, as a complete ration.

Before being supplied to the animals, the amount of daily silage to be fed was exposed to oxygen to allow the volatilization of ammonia. Leftovers were weighed daily, and the total amount supplied was adjusted to allow for 10% as leftovers, so as not to limit maximum voluntary intake. Also, it was evaluated during the feedlot the pH of the silages offered to the animals (Table 1).

During the experimental period, samples of roughage, ingredients and leftovers were harvested weekly, packed in plastic bags and stored in a freezer at −20°C. After thawing, samples were predried in a forced-air oven at 55°C for 72 hr and then ground through a Wiley mill with a 1-mm sieve and stored in labelled plastic bottles with lid for chemical analyses (Table 1).

Analyses for the chemical composition of the ingredients were performed to determine the dry matter (DM–method 967.03), mineral mater (MM–method 942.05), ether extract (EE–method 920.29) and crude protein (CP–method 981.10) contents, following methodologies described by AOAC (1990).

The neutral (NDF) and acid (ADF) detergent fibre contents were analysed according to Van Soest, Robertson, and Lewis (1991). For the ash correction, the residue from sample digestion in neutral detergent was incinerated in a muffle furnace at 550°C for 2 hr. For the protein correction, the residue underwent a crude protein analysis, subtracting the neutral detergent insoluble protein (NDIP).

The nonfibrous carbohydrates content of the ingredients was determined following Mertens (1997), considering the NDF value corrected for ash and protein in the calculation. Neutral (NDIP) and acid (ADIP) insoluble protein contents were obtained according to Licitra, Hernandez, and Van Soest (1996). Lignin was determined by treating the acid detergent fibre residue with 72% sulphuric acid, as described in Van Soest and Wine (1967).

The total digestible nutrients (TDN) content of the ingredients and diets were calculated according to the equation TDN = adNFC% + adEE% + adCP% + NDFdVL%, in which the apparently digestible NFC content (adNFC) was estimated by the equation (Detmann, Pina et al., 2006): adNFC% = 0.9507NFCap%−5.72 for lactating cows. The apparently digestible EE content (adEE%) was estimated by the equation (Detmann, Valadares Filho, Pina et al., 2006): adEE% = 0.8596EE%−0.21, for lactating cows. The

apparently digestible CP content (adCP%) was estimated by the equation (Detmann, Valadares Filho, Henriques et al., 2006): adCP% = 0.7845CP%−0.97, for lactating cows. The concentration of neutral detergent fibre corrected for ash and protein effectively digestible for lactating cows was estimated according to Detmann et al. (2007), as: NDFdLC% = 0.67 × {(NDFap−L) × [1−(L/NDFap)0.85]}, where NDFap is the neutral detergent fibre corrected for ash and protein and L is the lignin content (% DM).

The intake of nutrients was estimated as the difference between the total amount of each nutrient that was contained in the offered feed and the total amount of each nutrient that was contained in the orts. So, total dry matter intake was determined multiplying the mean value of intake of the animals which was multiplied by the experimental period (52 days).

The production performance of the animals was determined by taking into consideration the difference between the weights of all lambs obtained at the beginning and end of the experimental period. The animals were weighed after being subjected to a solid-food fasting period of 16 hr. Feed conversion (feed:gain) was obtained as the average dry matter intake of the lambs (kg/animal/day) divided by their body weight gain and expressed as kg of feed/kg of body weight gain. Average daily gain (ADG) was calculated as the difference between final and initial body weights of the animals divided by the number of days in the experimental period (52 days) and expressed in kg/day.

2.3 | Slaughter

After the end of the experimental period, lambs were deprived of solid feed and kept on a water-only diet for 16 hr. Afterwards, they were weighed to determine their body weight at slaughter (BWS) and then stunned by concussion in the atlanto-occipital region, followed by bleeding for three minutes by sectioning their carotid artery and jugular vein. Before decapitating the animals, we determined their body condition score, which was assessed subjectively by palpating the lumbar region, assigning scores of 1–5 (1 = poor, 5 = excellent).

After skinning and evisceration, we removed their head (by sectioning the atlanto-occipital joint) and feet (metacarpal and metatarsal joint section) and determined the hot carcass weight (HCW), with which we calculated the hot carcass dressing (HCD), by the following formula: HCD = HCW/BWS × 100. In a subsequent way, carcasses were cooled for 24 hr at 4°C and weighed again to determine the cold carcass weight (CCW) and calculate the cold carcass dressing (CCD = CCW/BWS × 100).

2.4 | Evaluation of carcass traits and sampling procedures

After being chilled overnight, carcasses were removed from the cold chamber and a subjective evaluation was performed according to the methodology mentioned by Cezar and Sousa (2007), considering the following variables: fatness–determined by a visual assessment using a scale of 1–5, where 1 = too lean and 5 = fat; carcass conformation–visual assessment of the carcass, considering the different anatomical regions (leg, rump, loins and shoulder) and the thickness of their muscle and adipose layers relative to the size of the skeleton, which supports them, assigning scores of 1–5, where 1 = very poor and 5 = excellent.

In a subsequent way, the carcasses were sawn lengthwise into two equal parts and sectioned into five anatomical regions: neck, shoulder, ribs, loin and leg, according to methodology adapted from and Silva Sobrinho (1999). A transverse section was made between the 12th and 13th ribs in the left half-carcass to measure the loin-eye area (LEA) of the *Longissimus lumborum* (*L. lumborum*) muscle, using a transparency with checkerboard pattern in which each square represented one square centimetre, according to the methodology of Yáñez et al. (2006). To measure the backfat thickness in the *L. lumborum* samples, we used a digital caliper on the muscle section (between the last thoracic vertebra and first lumbar) at two-thirds of the total loin-eye area length.

2.5 | Physicochemical analysis

Samples of the *L. lumborum* muscle from the left side of the carcass of each animal were used in analyses of meat centesimal composition and physicochemical properties, which were performed at the Laboratory of Animal Products (LAPOA), Center for Agricultural Sciences, Federal University of Paraíba (CCA/UFPB), located in Areia-PB, Brazil.

Cooking losses, shear force and colour of meat were determined by following the methodology described by Wheeler, Cundiff, and Koch (1995). Cooking loss was measured in samples previously thawed for 24 hr under refrigeration (4°C) and cut into 2.5-cm-thick steaks. After weighing, steaks were cooked in a preheated oven at 200°C until their geometric centre reached 70°C, with temperature monitored using a special thermometer for meat cooking (Acurite®). After cooking, samples were cooled at room temperature and weighed again. Cooking loss was calculated as the difference in weight of samples before and after cooking and expressed as a percentage.

A minimum of two cylindrical samples were taken from cooking samples left from the procedure for determination of cooking losses, using a 1.27-cm-diameter punch in the longitudinal orientation of the fibres, to determine shear force. The texture instrumental analysis was performed using a Warner-Bratzler Shear Force device (3000, G-R Manufacturing CO) with a 25-kgf load cell and at a crosshead speed of 20 cm/min, and shear forced was determined as the force necessary to transversally shear the muscle fibres of each cylinder. The average shear force values of each cylinder, expressed in kilograms force, were used to obtain an average to represent the toughness value of each sample.

Colour was determined after standardizing the cuts to a thickness of at least 15 mm, followed by exposure in air for 30 min in a refrigerated environment (4°C). This procedure is important in that it is through it that the reaction between the muscle myoglobin and

the oxygen in the air occurs forming oxymyoglobin, the main pigment responsible for the bright red colour of meat (Renerre, 1982). The meat colour was read using a chroma meter (CR-400, Konica Minolta), adopting the CIELAB system (L*, a*, b*), to determine the following parameters: L* (lightness), a* (redness) and b* (yellowness). Three measurements were performed in different points of the muscle, to obtain the mean values for colour of each animal.

2.6 | Proximate composition analysis

The meat centesimal composition was analysed using the *L. lumborum* muscle, which was ground and homogenized in a blender and lyophilized for later determinations of moisture (method 985.41), crude protein (method 928.08) and ash (method 920.153), following the methodology described by AOAC (2000). Lipids extraction was performed using Chloroform:Methanol (2:1, v/v) and was carried out following the method of Folch, Lees, and Sloane-Stanley (1957).

2.7 | Fatty acid profile analysis

Fatty acid methyl esters were analysed in a gas chromatograph (GC-17A, Shimadzu) equipped with a flame ionization detector, split/splitless injector and fused silica capillary column containing polyethylene glycol as stationary phase (DB-Wax, 60 m × 0.25 mm, J & W Scientific), under the following chromatographic conditions: injector temperature 230°C; the initial column temperature was 80°C for 2 min at a rate of 3°C per minute, which was then elevated to 180°C at a rate of 30°C/min, held at that temperature for 30 min and then increased to 200°C at a rate of 3°C/min, at which temperature it remained for 108 min. Detector temperature 240°C, helium as carrier gas with a total flow of 8.0 mL/min; sample split ratio 1:50.

For the identification of fatty acids, retention times were compared with the methyl ester standards (Sigma-Aldrich) of fatty acids containing the linoleic acid isomers cis-9 and trans-11 and trans-10 cis-12 (189-19, O-5632 and O-5626, Sigma, USA), whereas quantification was performed by normalizing the area, with results expressed as a percentage of area of each acid over the total fatty acid area (%), following the methodology described by Hartman and Lago (1973).

The nutritional quality of the lipid fraction was evaluated by two indices based on fatty acid composition data, by the following calculations: Atherogenicity Index (AI) = [(C12:0 + (4 × C14:0) + C1 6:0)]/(ΣMUFA + Σω6 + Σω3), according to Ulbricht and Southgate (1991); and ratio between hypocholesterolaemic and hypercholesterolaemic fatty acids (h:H) = (C18:1cis9 + C18:2ω6 + C20:4ω6 + C18:3ω3 + C20:5ω3 + C22:5ω3 + C22:6ω3)/(C14:0 + 16:0), according to Santos-Silva, Bessa, and Santos-Silva (2002), where MUFA = all monounsaturated fatty acids.

2.8 | Statistical analysis

A randomized block design with four treatments (levels of urea addition to the pearl millet silage) and eight replicates per treatment (lambs) was adopted, with blocks corresponding to the body weight intervals, according to the mathematical model below:

$$\gamma ij = \mu + \tau i + \beta j + \epsilon ij$$

where γij = value observed in the plot that received treatment i in block j; μ = overall mean; τi = effect of treatment i; βj = effect of block j; and εij = random error associated with treatment i and block j.

Results were subjected to analysis of variance (ANOVA) and regression at the 5% probability level, and the effect of treatments was evaluated by orthogonal polynomials, by decomposing the sum of squares into linear and quadratic effects, using the Statistical Analysis System (SAS 9.1®, 2003). The regressions equations were described in the manuscript, where "x" is the independent variable related to the g/kg of urea addition in pearl millet silage) and "Ŷ" is the dependent variable evaluated.

3 | RESULTS

Although no effects of ensiling pearl millet with urea were on feed conversion were observed ($p > 0.05$), diets promoted decrease in average daily gain ($p < 0.05$) (Ŷ = 140.5–5,25x; $R^2 = 0.71$), which may justify the effect on carcass traits in this study (Table 2).

Slaughter weight (Ŷ = 23.958–0.5334x; $R^2 = 0.88$) and hot and cold carcass weights (Ŷ = 11.555–0.3776x; $R^2 = 0.94$/Ŷ = 11.308–0.3753x; $R^2 = 0.94$ respectively) and hot and cold carcass yields (Ŷ = 478.12–4.525x; $R^2 = 0.93$/Ŷ = 469,93–5.185x; $R^2 = 0.95$) decreased linearly ($p < 0.05$) with the use of diets containing pearl millet silage ammoniated with urea (Table 2).There was a linear decrease ($p < 0.05$) (Table 3) in conformation (Ŷ = 3.044–0.1005x; $R^2 = 0.91$) and degree of fatness (Ŷ = 2.987–0.0815x; $R^2 = 0.65$) of the carcasses, suggesting that as urea was added to the ensiling of pearl millet, there was an effect on the proportion of muscle in the carcasses. Furthermore, the existence of these differences might be attributed to the fact that we used crossbreed animals, which may carry heterogeneous genetic material with different phenotypic expressions. The carcass conformation is a subjective measurement that does not serve as an indicator of lean meat yield; however, this information is important from the commercial perspective. Despite the reduction of conformation values resulting from the higher urea levels in the pearl millet silage, all values observed in the animals fed the different diets are considered adequate.

Despite the effects of diets on fatness, this reduction was not sufficient to influence ($p > 0.05$) the subcutaneous fat thickness, likely because adipose tissue is not deposited in a similar manner in the sheep carcass. Neither, no effect of urea levels that were used in pearl millet silage was found ($p > 0.05$) on loin-eye area (LEA) of lambs.

Redness (a*), shear force and cooking loss were not influenced ($p > 0.05$) by the urea levels at ensiling, averaging 10.55, 3.26 and 363.7 g/kg respectively (Table 4). However, the

TABLE 2 Dry matter intake, carcass traits of lambs fed pearl millet silage ammoniated with urea

	Urea level (g/kg DM)					*p*-value*	
Item	0	20	40	60	*SEM*	Linear	Quadratic
Average daily gain (g/day)	134.00	136.00	127.00	102.00	0.007	0.048	0.208
Feed conversion[a]	5.95	4.51	5.51	5.67	0.256	0.121	0.302
Total dry matter intake (kg)	41.50	31.88	36.4	30.05	0.031	0.049	0.616
Slaughter Weight (kg)	23.90	23.32	21.13	21.07	0.577	0.049	0.823
Hot Carcass Weight (kg)	11.46	11.08	9.75	9.39	0.263	0.003	1.000
Cold carcass Weight (kg)	11.20	10.84	9.54	9.13	0.319	0.004	0.964
Hot Carcass Yield (g/kg)	475.2	473.8	459.1	450	3.93	0.027	0.664
Cold Carcass Yield (g/kg)	467.2	463.8	448.9	437.6	4.40	0.014	0.660

Notes. SEM: standard error of the mean.
[a](kg of feed/kg BW gain); *significant at the 5% probability level.

TABLE 3 Qualitative characteristics of lambs fed pearl millet silage ammoniated with urea

	Urea level (g/kg DM)					*p*-value*	
Item	0	20	40	60	*SEM*	Linear	Quadratic
Carcass conformation (1–5)	3.12	2.75	2.60	2.50	0.092	0.020	0.450
Fatness (1–5)	2.84	3.03	2.69	2.41	0.082	0.034	0.167
Loin-eye area (cm^2)	10.22	10.84	9.78	10.75	0.226	0.795	0.708
Fat thickness (mm)	0.22	0.21	0.19	0.16	0.016	0.157	0.849

Notes. SEM: standard error of the mean.
*Significant at the 5% probability level.

TABLE 4 Physicochemical characteristics and proximate composition of *Longissimus lumborum* from lambs fed pearl millet silage ammoniated with urea

	Urea level (g/kg DM)					*p*-value*	
Variable	0	20	40	60	*SEM*	Linear	Quadratic
Physicochemical characteristics							
Lightness (L*)	34.74	34.57	38.28	37.16	0.490	0.019	0.631
Redness (a*)	10.13	10.54	11.34	10.18	0.212	0.619	0.073
Yellowness (b*)	6.87	6.82	8.01	7.46	0.119	0.010	0.300
Cooking losses (g/kg)	333.1	357.5	395.1	369.0	13.19	0.229	0.348
Shear force (N/cm^2)	260.9	358.9	277.5	382.4	0.241	0.193	0.950
Proximate composition (g/kg)							
Moisture	747.8	739.9	746.2	744.5	1.57	0.813	0.333
Protein	199	209.5	201.9	202.2	1.53	0.881	0.108
Fat	26.3	19.9	19.7	16.7	1.07	0.006	0.431
Ash	8.9	9.0	9.0	9.3	0.13	0.265	0.801

Notes. SEM: standard error of the mean.
*Significant at the 5% probability level.

increased urea levels provided a linear increase ($p > 0.05$) in lightness (L*) ($\hat{Y} = 34.542 + 0.5485x$; $R^2 = 0.60$) and yellowness (b*) ($\hat{Y} = 6.846 + 0.148x$; $R^2 = 0.46$), thus affecting the lamb meat colour (Table 4).

Based on the results for proximate composition of the meat (Table 4), there was no influence of the diets ($p > 0.05$) on moisture, protein, or ash contents, whose mean values were 744.6, 203.1 and 9.0 g/kg respectively. On the other hand, the fat content

(Ŷ = 24.965–1.44654x; R^2 = 0.86), however, decreased linearly (p < 0.05) with the increasing urea levels in the pearl millet silage. The reduced fat deposition resulting from the use of ammoniated silages, as well as the other variables that were influenced by the diets, are possibly related to the decreased nutrient intake. In this regard, it is possible that the animals extracted lower amounts of energy from the ammoniated silages, having lower slaughter weights and consequently lower fat deposition. Another factor that might explain this finding is that marbling fat is the latest to be developed. Therefore, the lambs underwent a feedlot period in which deposition of intramuscular fat might have still been ongoing.

No diet effect (p > 0.05) was observed on the moisture content of the meat although a significant result was expected, as this component is determined mainly by the fat content, which was in fact influenced by the treatments.

The fatty acid composition of the *L. lumborum* muscle of feedlot-finished lambs revealed that myristic (2.28/100 g), palmitic (29.23/100 g) and stearic (17.39/100 g) saturated; oleic monounsaturated (33.46 /100 g); and linoleic polyunsaturated (5.53/100 g of fatty acids were found in highest amounts, accounting for 87.89/100 g of the total fatty acids in lamb meat (Table 5).

Oleic (C18:1n9c) (Ŷ = 35.42–0.6525x; R^2 = 0.76) and erucic (C22:1n9) (Ŷ = 0.0314–0.0064x; R^2 = 0.71) fatty acids decreased linearly (p < 0.05), whereas linoleic (C18:2n6c) (Ŷ = 4.613 + 0.3065x; R^2 = 0.97) and eicosadienoic (C20:2) (Ŷ = 0.126 + 0.033x; R^2 = 0.88) acids increased with the urea levels added to the silage (Table 5) which can be a result of animal selectivity to the diets. The urea utilized as chemical additive in the pearl millet silage might have elevated the lipid metabolism in the rumen. Therefore, the meat fatty acid

TABLE 5 Fatty acid profile of the *L. lumborum* muscle of lambs fed pearl millet silage ammoniated with urea

	Urea level (g/kg DM)					*p*-value*	
Item g/100 g	0	20	40	60	*SEM*	Linear	Quadratic
C6:0	0.14	0.13	0.07	0.15	0.028	0.975	0.409
C8:0	0.25	0.21	0.19	0.24	0.017	0.768	0.195
C12:0	0.001	0.02	0.004	0.02	0.005	0.348	0.914
C13:0	0.14	0.16	0.18	0.15	0.011	0.731	0.261
C14:0	2.39	2.35	2.31	2.08	0.097	0.268	0.640
C16:0	29.71	29.16	29.38	28.67	0.391	0.416	0.917
C17:0	1.76	1.77	1.49	1.87	0.084	0.965	0.271
C18:0	17.39	16.77	16.89	18.5	0.485	0.433	0.264
C20:0	0.25	0.31	0.23	0.28	0.029	0.897	0.957
C21:0	0.005	0.02	0.00	0.00	0.005	0.493	0.405
C22:0	1.06	0.36	0.57	0.64	0.108	0.297	0.092
C23:0	0.11	0.01	0.00	0.004	0.026	0.195	0.405
C15:1	0.01	0.02	0.01	0.01	0.002	0.925	0.975
C16:1	2.60	2.67	2.57	2.50	0.082	0.581	0.672
C17:1	0.006	0.01	0.01	0.009	0.002	0.965	0.294
C18:1n9c	34.67	34.76	33.77	30.65	0.578	0.018	0.177
C18:1n9t	0.14	0.15	0.08	0.10	0.030	0.485	0.950
C20:1	0.42	0.50	0.53	0.56	0.026	0.071	0.698
C22:1n9	0.04	0.01	0.00	0.003	0.006	0.023	0.140
C18:2n6c	4.49	5.43	5.80	6.41	0.192	0.001	0.677
C18:2n6t	0.05	0.08	0.01	0.05	0.016	0.578	0.859
C18:3n3	0.46	0.51	0.51	0.76	0.077	0.215	0.513
C18:3n6	0.00	0.00	0.30	0.00	0.075	0.659	0.326
C20:2	0.12	0.22	0.22	0.34	0.024	0.008	0.799
C20:3n3	1.55	1.7	2.13	2.91	0.408	0.229	0.703
C20:4n6	2.09	2.21	2.49	2.86	0.169	0.100	0.712
C20:5n3	0.00	0.20	0.10	0.11	0.051	0.605	0.368
C22:6n3	0.00	0.007	0.02	0.009	0.006	0.453	0.392

Notes. SEM: standard error of the mean.
*Significant at the 5% probability level.

profile might have been changed, leading to a conversion of the monounsaturated (MUFA) into polyunsaturated fatty acids (Table 6). Based on the ratios or proportions of fatty acids in the meats from the studied lambs, there was a linear increase ($p < 0.05$) in monounsaturated fatty acids ($\hat{Y} = 38.23–0.6125x$; $R^2 = 0.66$), PUFA content ($\hat{Y} = 8.703 + 0.7615x$; $R^2 = 1.00$), PUFA:SFA ratio ($\hat{Y} = 0.163 + 0.0165x$; $R^2 = 1.00$) and ω6 content ($\hat{Y} = 6.665 + 0.4475x$; $R^2 = 0.99$).

On the other hand, a decreasing linear effect ($p < 0.05$) was observed on concentration of monounsaturated fatty acids (MUFA) as the urea levels were added in the ensiling of pearl millet (Table 6). In absolute terms, lambs that received the diet with 6% urea displayed high levels of SFA and low levels of oleic acid (C18:1), demonstrating that the use of urea at the level of 6% in the ensiling of pearl millet can reduce the meat quality of mixed-breed lambs.

4 | DISCUSSION

The main factors determining carcass qualitative and quantitative traits are sex (Koyuncu, Duru, Uzun, Özis, and Tuncel (2007), age (Galvani et al., 2010), genotype (Gomes et al., 2011), production system (Silva Sobrinho & Silva, 2000) and diet (Casey & Webb, 2010). In the present study, the only factor that could lead to a decrease in the slaughter weight and hot and cold carcass weights and yields would be the diet, as the other parameters were not changed with the different treatments. Therefore, the higher values obtained in lambs fed the nonammoniated silage may be attributed to their greater feed intake as compared with those receiving the diet ammoniated with 6% urea. Because of the observed reduction in dry matter intake, there was a decrease in weight gain that ultimately led to a decrease in carcass parameters.

Nevertheless, the values obtained for cold and hot carcass yields corroborate Silva Sobrinho (2001), who reported 460 and 445 g/kg for these variables. These findings suggest that pearl millet ammoniated with urea provides adequate body development when supplied in diets for finishing lambs and thus has the potential to be used as an alternative feedstuff.

Although the diets were isonitrogenous, DM intake decreased, and consequently the intake of CP by the animals in this study also declined. A decrease would thus be expected in LEA, as CP intake was compromised, and this nutrient is directly related to muscle tissue formation.

The subjective measurements were possibly attributed to the low acceptability of the diets with ammoniated pearl millet silage, which led to lower live weights at slaughter compared with the other diets. Thus, the values obtained of these measurements in the current study corroborates with Silva et al. (2012), who described that the carcass conformation is closely related to the body condition, and as the slaughter weight decreases, conformation values will decrease as well.

According to Cezar and Sousa (2010), the fat deposition pattern in the sheep carcass is distributed as follows: subcutaneous (300–440 g/kg), intermediate (420–340 g/kg intermuscular and 150–90 g/kg intramuscular) and internal (130 g/kg) fat. The authors also emphasized the difference in sequential order of deposition of adipose tissue in the sheep carcass, with the renal and pelvic fats being earlier; subcutaneous and intermuscular fats intermediate; and marbling fat the latest. In this way, in the stage at which the lambs were

TABLE 6 Ratios among saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in meat from lambs fed pearl millet silage ammoniated with urea

	Urea level (g/kg DM)					*p*-value*	
Item g/100 g	0	20	40	60	*SEM*	Linear	Quadratic
Saturated	52.34	51.10	51.09	52.12	0.620	0.904	0.370
Monounsaturated	37.26	37.93	36.84	33.54	0.515	0.013	0.065
Polyunsaturated	8.70	10.32	11.57	13.36	0.530	0.003	0.934
MUFA:SFA	0.71	0.74	0.72	0.65	0.016	0.150	0.107
PUFA:SFA	0.16	0.20	0.23	0.26	0.012	0.008	0.965
ω6	6.55	7.67	8.58	9.23	0.295	0.002	0.694
ω3	1.99	2.40	2.76	3.78	0.407	0.129	0.713
Desirable fatty acids [a]	63.06	64.93	65.23	65.18	0.436	0.100	0.284
Atherogenicity index [b]	0.84	0.80	0.80	0.80	0.021	0.485	0.638
Thrombogenicity index [c]	1.06	1.16	1.06	1.31	0.081	0.384	0.648
h:H[d]	1.35	1.41	1.42	1.44	0.036	0.423	0.765
(C18:0 + C18:1): C16:0	1.76	1.77	1.73	1.72	0.027	0.584	0.894

Notes. SEM: standard error of the mean.

*Significant at the 5% probability level;[a]Desirable fatty acids = MUFA + PUFA + C18:0; [b]Atherogenicity index = {(C12:0 + (4 × C14:0) + C16:0)}/(ΣMUFA + Σw6 + Σω3); [c]Thrombogenicity index = (C14:0 + C16:0 + C18:0)/{(0.5 × ΣMUFA) + (0.5 × Σw6 + (3 × Σω3) + (Σω3/Σw6)}; [d]Hypocholesterolaemic:hypercholesterolaemic fatty acid ratio = (C18:1cis9 + C18:2ω6)/(C14:0 + 16:0).

slaughtered, they were possibly still depositing intermuscular fat, at which point subcutaneous fat deposition had not yet occurred.

Moreover, another factor that was possibly relevant to promote the reduction in fatness is the decrease in dry matter intake by the animals and therefore the total energy consumed noticed in the present study. Increasing levels of food intake, as described by Geay and Robelin (1979), favours not only body fat deposition, but also, intramuscular lipids. Thus, the decreasing on fatness noticed corroborate with Geay, Bauchart, Hocquette, and Culioli (2001), as the muscle characteristics at slaughter depends not only on breed, sex and age of the animals but also on animal breeding conditions. So, the nature and the level of dietary intake is the reason why a high dietary intake is extremely important.

The results obtained in the current study related to the colour of lamb meat corroborate the literature on sheep meat, as these values are considered normal, ranging from 30.03 to 49.47, 8.24 to 23.53 and 3.38 to 11.10 for L*, a* and b* respectively (Warris, 2003). As stated by Pinheiro, Silva Sobrinho, Souza, and Yamamoto (2009), the meat colour is influenced by lightness and red intensity, whereas the yellow intensity is more significant for the fat colour. Moreover, the existence of marked differences between the results obtained in this study can be attributed to the fact that the animals used here were young, mixed-breed, and had different slaughter weights.

Meat with tenderness and shear force scores above 50 N/cm^2 is regarded as tough (Davey, Gilbert, & Carse, 1976) and will be discounted by consumers. In this study, the lamb meat can be considered very tender or tender, as the average values were below what was described by the aforementioned authors.

Determining the cooking loss is a step of extreme importance, because the water content retained after cooking is one of the main attributes associated with the meat juiciness (Hedrick, Aberle, Forrest, Judge, & Merkel, 1994). The lack of exudation problems can once again be explained by the pH values, as no pH lower than 5.4 or higher than 6.0 was found in the meat samples. According to Cezar and Sousa (2007), meats with these pH values would be characterized as being anomalously PSE (pale, soft, exudative) and DFD (dark, firm, dry) respectively.

Except for fat content, our results agree with Prata and Fukuda (2001), who described the chemical composition of sheep meat (mean values) as 750 g/kg of moisture, 190 g/kg of protein, 40 g/kg of fat, 11 g/kg of mineral matter, and less than 10 g/kg of carbohydrates. According to Geay et al. (2001), the crude protein content of ruminant meats ranges from 11% to 22% in the fresh matter.

The amount and the nature of lipids stored in muscle, according to Hocquette and Bauchart (1999) mainly depends on feed conditions, and on digestion, intestinal absorption, hepatic metabolism and lipid transport systems to muscle. In addition, the authors mentioned that in the weaned ruminant, a high proportion of dietary unsaturated fatty acids (FA) is hydrogenated in the rumen, leading to intramuscular fatty acids that are far less unsaturated in bovines and ovines species compared to pigs and poultry.

Various factors, such as breed (Zygoyiannis, Kufidis, Katsaounis, & Phillips, 1992), weight (Kemp, Mahyuddin, Ely, Fox, & Moody, 1980; Velasco et al., 2000), degree of fatness (Kosulwat, Greenfield, & James, 2003), gender and diet (Solomon, Lynch, Paroczay, & Norton, 1991) may affect the fatty acid composition of lamb fat.

Since the diets in the present study promoted different weight gains in the animals and, consequently, differences in carcass weights and yields, this may have caused a difference in the deposition of fatty acids in the lambs′ meat. This behaviour corroborates with Duncan and Garton (1967) who mentioned that fatty acid composition can also vary between various sites and fat depots in the body of farm animals.

In general, there is a progressive increment in fatty acid saturation from peripheral to deep sites in farm animals (Casey & van Niekerk, 1985; Potchoiba, Lu, Pinkerton, & Sahlu, 1990; Wood, 1984). Studies conducted by Kosulwat et al. (2003) in weaned light lambs demonstrated that the proportion of myristic acid improved and stearic acid reduced as carcass fatness increased. Similar to that, Zygoyiannis et al. (1992) also reported that stearic acid concentrations decreased while proportions of all other fatty acids increased in fat depots of unweaned kids as slaughter age and, thus, fatness increased.

Higher concentrations of myristic, palmitic, stearic, oleic and linoleic fatty acids in lamb meat were also found by Perez et al. (2002), Demirel, Ozpinar, Nazli, and Keser (2006), Nuernberg, Fischer, Nuernberg, Ender, and Dannenberger (2008) and Leão et al. (2011), who evaluated the effect of different types of diets on the fatty acid profile of lamb. Along with the observed decrease, the elevated concentration of oleic acid found in this study corroborates the literature regarding its presence in the composition of intramuscular fat in ruminants (Sañudo et al., 2000). According to the Centre d'Information des Viandes (CIV, 1996), muscle fatty acid in bovine and lamb are composed of 50% saturated FA (SFA) and 50% unsaturated FA, being the most abundant FA the oleic acid. Thus, this fatty acid is present in ruminant meat, originates from the intense incomplete biohydrogenation of unsaturated fatty acids, and particularly from the conjugated linoleic acids of the diet (French et al., 2000). However, long-chain fatty acids, such as C20 and C22 (ω3), are not prone to being modified by rumen micro-organisms (Ponnampalam, Sinclair, Egan, Blakeley, & Leury, 2001). The results obtained here corroborate those found by these authors, as we found increased deposition of polyunsaturated fatty acids (C18:2n6c, C20:2) in the muscle, which improves the meat nutritional quality.

The fatty acid composition of forages is formed in a larger proportion by the linolenic and linoleic polyunsaturated acids (C18:2) (Harfoot & Hazlewood, 1988), but also in small quantities by oleic acid (C18:1) (Harfoot, 1981). With respect to forages preserved as silage, however, C18:3 was present in small portions as an esterified fatty acid, with no complex lipids observed. The concentration of fatty acids in forage plants depends on several factors, including species and senescence (Bauchart, Doreau, & Legay-Carmier, 1985; Harfoot & Hazlewood, 1988; Harwood, 1980), growth stage, preservation method (Lough & Anderson, 1973; Yang & Fujita, 1997), as well as wilting, shading, and use of ensiling additives (Dewhurst & King, 1998).

In silages, the majority of fatty acids are present in the form of free fatty acids (FFA), due to lipolysis (Steele & Noble, 1984). Because lipolysis is a prerequisite for rumen biohydrogenation, a high amount of FFA in the forage may result in a high rate of biohydrogenation. Lipolysis in the silage can explain, at least partially, the lower concentration of polyunsaturated fatty acids (PUFA) in milk observed when forage is provided in preserved form rather than fresh (French et al., 2000). Thus, lower lipolysis during ensiling could result in lower rumen biohydrogenation.

The linear effect of diets on the polyunsaturated fatty acids may be related to the fact that the lambs selected the concentrate in the trough. As stated by Enser et al. (1998), linoleic acid is present in a larger amount in grains than in forages, and diets with greater proportions of concentrate produce animals with a higher unsaturated lipid profile.

Ponnampalam et al. (2001) asserted that the roughage source contains higher levels of linolenic acid (C18:3), a precursor of the ω3 fatty acid series, while the concentrate contains higher levels of linoleic acid (C18:2), a precursor of the ω6 series. This finding was noted in the current study, in which silage intake decreased as the urea levels in the silage were increased, which may be a result of the lambs having selected the concentrate in the trough.

No influence of the diets ($p > 0.05$) was detected on the PUFA:MUFA, MUFA:SFA, h:H, ω6:ω3 and (C18:0 + C18:1):C16:0 ratios or on desirable fatty acids (DFA) content, AI, TI and h:H (Table 6). These ratios or proportions between fatty acids have been studied for an analysis and identification of the sheep meat risk factor for increased blood cholesterol in humans.

As stressed by Wood et al. (2004), the UK Department of Health recommends that the PUFA:SFA ratio of a food be greater than 0.4 so as to prevent diseases associated with consumption of saturated fats. In the present study, despite the elevation in this ratio, the obtained result cannot be considered satisfactory, as it is below the recommended level.

Except for the urea level of 6%, the lamb meats showed lower thrombogenicity indices (TI) than the maximum ideal value of 1.27 proposed by Ulbricht and Southgate (1991). The atherogenicity index (AI) values, however, were above the ideal standard, which must not exceed 0.72. The atherogenicity and thrombogenicity indices, as well as the ratio between hypo- and hypercholesterolaemic acids, are of extreme importance in the characterization of the nutritional value of a feedstuff, because they indicate how harmful it is, or can be, to human health. According to Turan, Sönmez, and Kaya (2007), the indices previously mentioned indicate potential stimulus to platelet aggregation; in this way, as the AI and TI values decrease, the amount of anti-atherogenic fatty acids present in the fat increases and consequently so does the potential for prevention against appearance of coronary diseases.

5 | CONCLUSIONS

Pearl millet silage ammoniated with urea allows for the production of good-quality lamb meat, with greater concentrations of polyunsaturated fatty acids. However, lambs fed diets with pearl millet ammoniated with up to 6% lead to a reduction of carcass characteristics without significantly affecting loin-eye area possibly associated with low palatability of the additive used.

The lower acceptance of the silages with higher levels of urea is due to the ammonia retention in the material is attributed to the ammonization process. Thus, in spite of the benefits on lamb meat quality, it is suggested that the use of this additive in the ammonization of tropical forages be carried out with care, in limits of up to 6%.

ORCID

Gleidson G. P. de Carvalho http://orcid.org/0000-0002-4108-6782

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How to cite this article: Carvalho GGPD, Freitas PMDD, Santos EM, et al. Effects of pearl millet silage ammoniation with urea on carcass and meat quality of lambs. *J Anim Physiol Anim Nutr*. 2018;102:1154–1166. https://doi.org/10.1111/jpn.12945