UNIVERSIDADE FEDERAL DA BAHIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

PHASE-FEEDING CRUDE PROTEIN IN FEEDLOT DIETS FOR CROSSBREED SANTA INES LAMBS

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Dissertação parcial apresentada ao Programa de Pós-Graduação em Zootecnia da Universidade Federal da Bahia como requisito para obtenção do título de mestre em Zootecnia.

Área de Concentração: Produção e Nutrição de Ruminantes

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Dedico a Deus pela graça e misericórdia, aos meus pais e irmão amado, pela força e apoio incondicional...

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“Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito.
Não sou o que deveria ser, mas graças a Deus, já não sou o que era antes.”

Marthin Luther King

BIOGRAFIA

CINTIA RAQUEL NUNES DE OLIVEIRA, filha de Sulineide Nunes de Oliveira e José Acácio de Oliveira, nasceu em Juazeiro, Bahia, em 30 de outubro de 1991. Concluiu o Ensino Médio no Colégio Modelo Luís Eduardo Magalhães – CMLEM/Juazeiro/BA, em 2008.

Concluiu a graduação em Zootecnia no ano de 2014, pela Universidade Federal do Vale do São Francisco – UNIVASF/Petrolina/PE. Durante a graduação, desenvolveu pesquisas como discente de iniciação científica da UNIVASF e CNPq, nas áreas de Reprodução animal, Extensão rural com ênfase em agroecologia.

Ingressou no Programa de Pós-graduação em Zootecnia (Área de concentração: Produção nutrição de ruminantes) em março de 2015, da Universidade Federal da Bahia – UFBA, em Salvador/BA, como bolsista da FAPESB, sob orientação da Profª Drª Stefanie Alvarenga Santos, com a Dissertação intitulada: “Phase-feeding crude protein in feedlot diets for crossbreed santa ines lambs”. Em março de 2017, submeteu-se à defesa da dissertação de mestrado.

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RESUMO

Dois experimentos foram conduzidos para avaliar os efeitos da redução do teor de proteína bruta (PB) na dieta e também os efeitos da alimentação alternada de PB nas dietas sobre a ingestão de nutrientes, digestibilidade aparente, fermentação ruminal, excreção e retenção de nitrogênio (N), síntese microbiana de proteínas, desempenho animal, características de carcaça e composição de ácidos graxos do tecido muscular de ovinos Santa Inês. Utilizaram-se quarenta ovinos cruzados Santa Inês, com dois meses e peso corporal inicial de 15,6 ± 1,6 kg no primeiro ensaio. O período experimental durou 70 dias, divididos em dois períodos de 35 dias cada. A fase de alimentação inicial (IFF) ocorreu durante os primeiros 35 dias do período de confinamento, em que todos os animais foram distribuídos aleatoriamente para dois níveis de proteína bruta (PB) na dieta (130 ou 150 g / kg MS). A fase de alimentação final (FPF) ocorreu entre o 36º ao 70º dia de confinamento, e nesta fase, metade dos animais alimentados com cada um dos conteúdos iniciais de PB foram aleatoriamente designados para reversão do nível de PB na dieta. No segundo ensaio, quatro ovinos machos mestiços Santa Ines, com quinze meses e BW inicial de 60,6 ± 10,6 kg kg foram distribuídos aleatoriamente em oito quadrados latinos 2 x 2. Cada período consistia em oito dias para adaptação dos animais e seis dias para coletas. As dietas experimentais consistiram em dois teores de PB (130 ou 150 g PB / kg MS). Em ambos os ensaios, as dietas consistiram em 50% de feno de Tifton e 50% de concentrado. No primeiro ensaio, não houve efeito (P> 0,05) do teor de PB no IFP e FPF na ingestão de nutrientes, com exceção da PB. A ingestão de PB (P <0,05) foi maior para animais alimentados com 150 g / kg de PB do que aqueles alimentados com 130 g / kg. Não houve efeito (P> 0,05) do conteúdo dietético de PB em IFP e FPF na digestibilidade da MS e nos outros constituintes da dieta. A excreção urinária N (P = 0,01) aumentaram em resposta a PB dietética no IFP e FPF, enquanto que

no FPF o conteúdo PB não afetou o N fecal (0,72), retenção de N (P = 0,17), N retido: N ingerido (P = 0,94) E N retido: N absorvido (P = 0,31). A síntese de PBM e a eficiência microbiana não foram afetadas (P> 0,05) pelos teores de PB em IFP e FPF. Os ovinos apresentaram ganho médio diário semelhante (GMD, P = 0,48), peso corporal final (P = 0,65) e espessura de gordura subcutânea (P = 0,58). No segundo ensaio, os ovinos alimentados com 150 g / kg de MS apresentaram maiores concentrações ruminais de nitrogênio amoniacal (P <0,01) e N-uréico sanguíneo (P <0,01) do que aqueles alimentados com 130 g / kg MS. O conteúdo de CP não afetou a produção de ácido acético (P = 0,95), ácido propiónico (P = 0,26) e ácido butírico (P = 0,95). Nossos resultados sugerem que não há benefício no uso de dietas com níveis proteicos acima de 130 g / kg MS e nem o uso de alimentação de PB por fases, para ovinos Santa Inês em crescimento.

Palavras-chave: Amônia, digestibilidade, eficiência, excreção, ácidos graxos, nitrogênio.

ABSTRACT

Two studies were conducted to evaluate the effects of reducing dietary crude protein (CP) content and also the effects of phase-feeding of CP in diets on nutrient intake, apparently digestibility, ruminal fermentation, nitrogen (N) excretion and retention, protein microbial synthesis, animal performance, carcass attributes and fatty acids composition of muscle tissue of Santa Ines sheep. Forty Santa Ines crossbred sheep, with two months and initial body weight (BW) of 15.6 ± 1.6 kg were used in the first trial. The experimental period lasted 70 days, divided in two periods of 35 days each. The initial feeding phase (IFF) occurred during the first 35 days of confinement period, in which all animals were randomly assigned to two crude protein (CP) content in the diet (130 or 150 g/kg CP, based on dry matter (DM)). The final phase feeding (FPF) occurred between the 36^{th} to the 70^{th} day of confinement. In this phase, half of the animals fed with each of the initial CP content were randomly assigned for reversion of the CP level in the diet. In the second trial, four Santa Ines crossbred sheep, male, with fifteen months and initial BW of 60.6 ± 10.6 kg kg were randomly allocated in eight 2 × 2 Latin squares. Each period consisted of eight days for adaptation of the animals and six days for sampling. Experimental diets consisted of two CP contents (130 or 150 g CP/kg DM). In both trials, the diets consisted of 50% Tifton hay and 50% concentrate. In the first trial, there was no effect ($P > 0.05$) the CP content in IFP and FPF on nutrients intake, by exception CP. CP intake ($P < 0.05$) was higher for animals fed with 150 g/kg CP than those fed with 130 g/kg. There was no effect ($P > 0.05$) of dietary CP content in IFP and FPF on digestibility of DM and on the other dietary constituents. The urinary N excretion ($P = 0.01$) increased in response to dietary CP, while that the FPF CP content did not affected the fecal N (0.72), N retention ($P = 0.17$), N retained:N ingested ($P = 0.94$) and N retained:N absorbed ($P = 0.31$) ratios. The MCP synthesis and the microbial efficiency were not

affected (P> 0.05) by CP contents in IFP and FPF. The sheep showed similar average daily gain (GMD, P = 0.48), final body weight (P = 0.65) and subcutaneous fat thickness (P = 0.58). In the second trial, the sheep fed with 150 g/kg CP had greater concentrations of ruminal nitrogen ammonia (P < 0.01) and blood N-urea (P < 0.01) than those fed with 130. CP contents did not affect acetic acid production (P = 0.95), propionic acid (P = 0.26) and butyric acid (P = 0.95). Our results suggest there is no benefit in using diets with great levels than 13% and nor in using phase-feeding of CP for Santa Ines sheep.

Keywords: ammonia, digestibility, efficiency, excretion, fatty acids, nitrogen

1. REFERENCIAL TEÒRICO

Segundo Valadares Filho et al. (2006) a proteína é o segundo nutriente que é mais exigido pelos ruminantes, quando comparado aos outros nutrientes que constituem a dieta. Sendo de grande importância para nutrição animal.

Os ruminantes possuem a capacidade de transformar nitrogênio (N) dietético de qualidade relativamente baixa em proteínas animais de alta qualidade (isto é, carne e leite). No entanto, apresentam baixa eficiência de utilização do nitrogênio (N), sendo que apenas de 20 a 30% do N ingerido é convertido em produto animal, e aproximadamente 70 a 80% é excretado na forma de fezes e urina (Wessels et al., 1997; Colle et al., 1999). O teor de N na dieta é um importante fator que influencia nas quantidades de N excretadas pelos animais (Waldrip et al., 2013).

Estudos com ovinos demonstram que, o aumento nos níveis de PB nas dietas proporciona maiores desempenhos (Zundt et al., 2002; Ortiz et al., 2005), porém o excesso de PB pode reduzir a eficiência de utilização do N através do aumento nas excreções dos compostos nitrogenados. Rocha et al., (2002) observaram que as excreções de N na urina em ovinos aumentaram de 7 g/dia para 14 g/dia em resposta ao aumento dos níveis dietéticos PB de 12 para 20%. Por outro lado, a deficiência de proteína pode comprometer a digestão ruminal através da limitação do crescimento microbiano e do desbalanço entre proteína e energia no rúmen (Clark et al., 1992; Reynal e Broderick, 2005) com consequente impacto negativo sobre o consumo.

Ressalta-se que o N excretado nas fezes e urina dos animais gera importantes implicações ambientais, podendo afetar a qualidade do ar e do solo. O N excretado na urina dos animais é de particular preocupação, devido à alta volatilização em amônia (NH_3) e óxido nitroso (N_2O), sendo estes gases reconhecidos como potenciais poluentes ambientais (Barrow et al., 1987; Bouwmen et al., 1997; Onema et al., 2005). Além do

mais, a proteína é o nutriente de mais alto custo unitário nas dietas de ruminantes, sendo que sua inclusão de forma desequilibrada resulta em elevação nos custos de produção (Cavalcante et al., 2005).

Portanto, ofertar PDR (proteína degradável no rúmen) em quantidades adequadas e, consequentemente, uma concentração adequada de N-NH3 (nitrogênio amoniacal) ruminal, é a primeira prioridade para a otimização da digestão fermentativa de forragem (Detmann et al., 2009). A utilização não eficiente da PDR impulsiona a transformação de N aminoacídico em N-NH3, e caso este não seja incorporado a proteína microbiana, será excretado na forma de ureia (Broderick et al., 1994).

As perdas de N ruminal ocorrem principalmente por desequilíbrio entre a degradação de substratos contendo N e a disponibilidade de N para os microrganismos, quando em altas concentrações no sangue, a amônia é tóxica para os ruminantes, sendo então convertida em ureia no fígado (ciclo da ureia). Rações com excesso de proteína levam a um excesso de amônia ruminal e requerem quantidades significativas de energia para síntese e excreção de ureia, uma vez que para cada mol de ureia produzido são gastos 2 moles de ATP (Santos, 2006).

Em ruminantes, há uma constante reciclagem de ureia entre o fígado e o trato gastrointestinal (TGI), um mecanismo que conserva N (Stewart e Smith, 2005), A reciclagem de ureia pode contribuir para o pool de amônia ruminal, produção microbiana e melhoraria da eficiência da conversão de alimentos para animais (Lapierre e Lobley, 2001). Portanto, entre 0,04 e 0,73% do N pode retornar ao intestino (Lapierre et al., 2005).

A capacidade dos microrganismos ruminais de sintetizar proteína através da ureia reciclada para o rúmen poderia, em teoria, compensar uma parte da perda potencial de N que ocorre através da degradação da proteína no rúmen e metabolismo de AA absorvido do intestino (Calsamiglia et al., 2010). A ureia que é reciclada para rúmen fornece uma

importante fonte de N degradável para o rendimento de proteínas microbianas, quando o fornecimento de N na dieta ou a degradação ruminal é deficiente (Lapierre e Lobley, 2001). Entretanto, essa reciclagem torna-se mais importante quando o N dietético fornecido é deficiente.

Assim é importante a adoção de estratégias que resultem em maior retenção de N, visando disponibilizar quantidades adequadas de N para o crescimento microbiano e produção animal, o que torna interessante a realização de estudos utilizando diferentes níveis de PB para se estabelecer o nível adequado na formulação das dietas.

Desta forma, baseando-se nas exigências dos animais, é interessante avaliar a possibilidade de reduzir as quantidades de PB nas dietas de ovinos, podendo potencialmente não somente minimizar os nutrientes excretados sem implicar em alterações no desempenho, mas também minimizar o impacto ambiental e reduzir o custo com as dietas, visto que a alimentação representa de 60 a 70% dos custos de produção (Martins et al., 2000).

Existe uma lacuna na nutrição proteica de ovinos em regiões tropicais, pois as exigências de PB dos ovinos não são bem estabelecidas, o que justifica a adoção do sistema americano National Research Council (NRC, 2007) como base para a formulação de dietas e determinação dos níveis de PB para ovinos, porém tal sistema estudou requerimentos de nutrientes utilizando raças que não são típicas do nosso país de clima quente, pois sabe-se que existem diferenças no potencial de crescimento entre as raças (Abdelrahman e Aljumahh).

O NRC (2007) recomenda o nível de 13% de PB dietética para o atendimento das exigências de um ovino para que este seja abatido com 30 Kg e para um animal pesando 20kg 19,6% de PB dietética, níveis estimados para um ganho médio de 200 g/dia. Porém, existe uma variação na literatura nacional atual quanto aos níveis proteicos

adotados, não sendo condizentes com o teor sugerido pelo NRC 2007, variando de 10% a 17% de PB nos trabalhos mais atuais, como mostrado no quadro a seguir:

% PB (MS da dieta)	Fonte
15%	Campos et al., (2017)
14%	Matoso Silva et al., (2016)
14% a 12%	Oliveira Filho et al., (2016)
16 a 17%	Oliveira et al., (2016)
16%	Pereira et al., (2016)
10 e 14,25%	Santos et al., (2015)
14,2 a 15,2%	Santos et al., (2016)

Sabe-se também que, as exigências de PB dos ruminantes alteram com o avanço da maturidade, ocorrendo um aumento na deposição da gordura em relação à deposição de proteína corporal dos animais (AFRC, 1993; Amaral, 2014). Isto demonstra que o N pode estar sendo fornecido nas dietas além dos requerimentos na fase final do confinamento (Klopfenstein et al., 2002). Pois, quando o suprimento proteico é limitante, a deposição de proteínas aumentará linearmente com o aumento da ingestão proteica (fase dependente de proteína), até que um ponto seja atingido onde a energia se torna mais limitante e o animal não responde mais ou responde apenas com uma baixa eficiência, a aumentos adicionais no fornecimento de proteínas (Schroeder et al., 2008).

Desta forma, existe a possibilidade de reduzir as concentrações de PB nas dietas de ovinos, alterando os níveis durante as fases de alimentação (crescimento e terminação) sem afetar o desempenho. Esta estratégia alimentar é reconhecida internacionalmente como “phase feeding”, sendo amplamente estudada em bovinos (Amaral et al., 2014; Cole et al., 2006; Cooper et al., 2000), essa estratégia consiste em alimentar os animais considerando as fases de produção. Portanto é importante a aplicação de estudos utilizando diferentes níveis de PB nas dietas, assim como estratégias que alterem os níveis nas fases de crescimento e terminação dos ovinos. De uma maneira geral, a redução no teor de proteína bruta na dieta para níveis abaixo de 120 g kg-1 MS não é

recomendada em dietas para ovinos em desenvolvimento, uma vez que a disponibilidade de nitrogênio poderá reduzir a digestão da fibra e, consequentemente, restringir o consumo (Roseler et al., 1993).

A proteína pode influenciar o consumo dos nutrientes, quando a suplementação de nitrogênio, não atende as exigências dos microrganismos do rúmen, podendo assim, limitar o crescimento microbiano e afetar negativamente a digestibilidade da parede celular; tendo em vista que a digestibilidade, é um importante indicativo do valor nutritivo da dieta e indica a porcentagem de cada nutriente utilizado pelo animal, quando a população microbiana é reduzida, consequentemente reduz a ação destes sobre o alimento. No entanto, níveis elevados de PB podem induzir um caso de toxicidade devido ao excesso de libertação de amônia, elevando assim suas concentrações sanguíneas. Isso ocorre quando a quantidade de amônia absorvida ultrapassa a capacidade do ciclo da ureia de metabolizar tal composto, a amônia tem grande capacidade de migrar para o interior das células, afetando principalmente o sistema nervoso (Minson, 1982; Van Soest, 1994). Além disso, como já descrito anteriormente, o excesso de amônia é excreto na forma de ureia.

A quantificação da síntese de PB microbiana é de grande interesse na nutrição dos ruminantes (Broderick e Merchen, 1992). Uma vez que, o nível da síntese da proteína microbiana a partir da amônia depende principalmente da disponibilidade de energia gerada pela fermentação de carboidratos (Broderick; Reynal, 2009).

As exigências dietéticas de proteína metabolizável (PM) para ruminantes são atendidas mediante a absorção no intestino delgado dos aminoácidos oriundos da PB microbiana e da proteína dietética não degradada no rúmen (PNDR) digestível (Valadares Filho et al., 2010).

A amônia ruminal resulta em parte do processo de degradação de PB no rúmen e representa a principal fonte de N utilizado para a síntese de PB microbiana. Portanto, diferentes níveis de PB na dieta influenciam na disponibilidade de N para o crescimento dos microrganismos e consequentemente no atendimento das exigências dos animais.

Uma ferramenta importante para análise do metabolismo proteico é a análise do balaço de nitrogenado que permite avaliar se o animal encontra-se em equilíbrio quanto aos compostos nitrogenados (Guimarães júnior et al., 2007).

Diante do que foi exposto, nota-se que existe uma necessidade de avaliar diferentes estratégias alimentares que concilie a produtividade animal com a otimização da eficiência na utilização dos compostos nitrogenados.

Diferentes respostas produtivas podem ser obtidas com alterações dos níveis de PB nas dietas. A otimização do balanço entre a síntese de PB microbiana e a degradação de proteína no rúmen pode ser obtido através do adequado fornecimento de PB para ovinos, resultando na maximização da captura de N pelos microrganismos ruminais e na taxa de crescimento destes, com consequente melhoria na eficiência animal. Portanto, entender como os ruminantes em crescimento respondem a variações no suprimento de nutrientes, é de fundamental importância para prever seu desempenho e melhorar a eficiência da utilização de nutrientes.

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3. INTRODUCTION

The cost of most protein sources commonly used in ruminant diets associated with the low efficiency to convert N intake in meat production, represent the main concerns in studies to meet optimal CP content in sheep diets. According the American system for small ruminants requirement, NRC (2007), crude protein (CP) content in diets for sheep with latter maturity, body weight among 20 and 30 kg, are wide range between 130 and 190 g of CP per kg to achieve average daily gain of 200 g/day.

In Brazil, there is a great preference in slaughtering of young sheep, being considerable the early feedlot of just-weaned lambs, starting at average 16 kg and slaughtering at 32 kg. Then, there is raising interest in studies evaluating this weight range. However, it is important to note that NRC (2007) reported nutrient requirements of breeds that are not typical in warm climate' countries, which could lead to inconsistencies with our conditions, because it is known there are differences in growth potential between different breeds (Abdelrahman and Aljumahh, 2014).

It is well established that protein requirements of sheep reduces in response to maturity, also when CP is excessive in ruminant' diets N excretion could increase and consequently efficiency of N usage for production could decrease. In this way, studies altering CP content during growing phase have been development to overcome these limitations. Hajji et al. (2015) varying CP between 110 and 160 g/kg in latter portion of feeding period for sheep (mean BW=41 kg), reported there was no difference in average daily gain (ADG) in response the increase CP increase.

Emon et al. (2011) reported that increases in DM intake using 200 g of CP/ kg for sheep, did not improve ADG when compared to those feed with 140 g of CP/ kg DM, suggesting there are no benefits when using high CP levels. Dabiri and Thonney (2004) obtained similar ADG when (ranging BW=25-40 kg) studied CP content ranging from

150 to 170 CP for growing sheep diets, which could lead to suppose that using above 150 g of CP/kg DM for young sheep do not improve animal performance. According to Amaral et al. (2014) phase-feeding scheme as a strategy for feedlot diets may decrease the nitrogen compounds excreted into the environment, depending mainly on the body weight and expected weight gain. Thus, we hypothesized that confined just-weaned Santa Ines lambs fed with 150 g/kg DM at growing phase and 130 g/kg DM in finishing phase improve N retention and productive performance in both phases. The objectives of this work were to evaluate the effects of reducing dietary CP content and also the effects of phase-feeding of CP in diets on nutrient intake, apparently digestibility, ruminal fermentation, nitrogen excretion and retention, protein microbial synthesis, animal performance, carcass attributes and fatty acids profile of muscle tissue of Santa Ines sheep.

4. MATERIAL AND METHODS

4.1. Trial 1 – Performance trial

All of the procedures with the animal's care and handling were approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Sciences of the Federal University of Bahia under protocol number 32-2016.

4.1.1. - *Animals, experimental design and diets*

This experiment was conducted at the experimental farm of the School of Veterinary Medicine and Animal Sciences of Federal University of Bahia, located at the municipality of São Gonçalo dos Campos, Bahia State, Brazil. All chemical analyzes were performed at the Laboratory of Animal Nutrition of the School of Veterinary Medicine and Animal Sciences of the same institution.

Forty Santa Ines crossbred sheep, male, uncastrated, with average body weight (BW) of 15.6 ± 1.6 kg, all at two months of age, were used. The animals were housed in individual metabolic cages with a total area of 1.3 m^2 totally covered, with slatted floor, equipped with individual feeders and water drinkers. The animals were submitted to a 15-d adaptation period, during which they were weighed, identified and dewormed.

The experimental period lasted 70 days, divided in two periods of 35 days each. The initial feeding phase (IFP) occurred during the first 35 days of confinement period, in which all animals were randomly assigned to two crude protein (CP) contents in the diet (130 or 150 g CP/kg DM, based on dry matter (DM)), totalizing 20 animals in each of the CP levels. That is, half of the animals were fed with 130 g CP/kg DM and another half were fed 150 g CP/kg DM. The final phase feeding (FPF) occurred comprising the following period between the 36^{th} to the 70^{th} day of confinement. Thus, on the 36th day of confinement, half of the animals fed with each of the initial CP content were randomly

assigned for reversion of the CP level in the diet. That is, half of the animals that were fed with 130 g CP/kg DM on IFP started to receive a diet with 150 g CP/kg DM on the FPF. The same changing was applied to the animals initially fed with 150 g/kg DM (Figure 1), which started to consume a 130 g CP/kg DM diet. The experiment was conducted in a completely randomized design, in a 2 × 2 factorial scheme, in which the factors were two CP contents in the IFP (130 or 150 g CP/kg DM, from days 1 to 35) and two CP contents in the FPF (130 or 150 g CP/kg DM, days 36-70).

The diet was formulated according to NRC (2007) to provide expected average daily gain of 200 g/d for growing sheep, with exception for CP content. The diets consisted of 50% tifton hay and 50% concentrate on DM basis. The proportion and chemical composition of feed used in experimental diets was shown in Table 1. The mineral mixture was composed by 147.0 mg/kg de sodium; 120 mg/kg de calcium; 87 mg/kg de phosphorus, 18 mg/kg de sulfur; 3.8 mg/kg de zinc; 1.8 mg/kg de iron; 1.3 mg/kg de manganese; 0.59 mg/kg de cupper; 0.3 mg/kg de molybdenum; 0.08 mg/kg de iodine; 0.04 mg/kg de cobalt; 0.02 mg/kg de chromium e 0.015 mg/kg de selenium.

Experimental diets were provided *ad libitum* twice a day at 8:00 a.m. and 16:00 p.m. in similar proportions. Intake has been adjusted to keep leftovers at maximum 10 to 20% of the offered daily amount on fresh basis.

4.1.2. *Experimental procedures and sampling*

Samples of the supplied hay and the leftovers of each animal were sampled daily during all experimental period and were weekly submitted to partial drying in a forced air oven (55 °C) for 72 hours. After drying, each sample was milled through 1 mm sieve in a knife mill (Wiley mill; TECNAL, São Paulo, SP, Brazil) and then proportionally composed (based on dry weight), per animal and per period. Hay samples were only

composed per period. The concentrate ingredients were sampled from the storage bags exactly in the days of the mixtures thereof. Samples were stored for further laboratory analysis.

From the 30th to the 34th day of each experimental period, fecal samples were collected for the determination of nutrient digestibility. All feces were collected during this four consecutive days (Lazzarini et al., 2016) in every four hours, directly from the collection bags adapted to the animals and later, they were packed in plastic containers with. At the end of each 24 hours fecal samples were weighed, homogenized and a daily sample was submitted to partial drying in a forced air oven (55 °C) for 72 hours. Afterwards the samples were milled with 1 mm sieves with a knife mill (Wiley mill; TECNAL, São Paulo, SP, Brazil) and proportionally composed (based on dry weight), per animal and per period.

From the 33rd to 34th day of each experimental period the animals were submitted to total urine collection for nitrogen balance and microbial CP (MCP) synthesis evaluation. Collecting funnels were connected to polyethylene hoses that carried urine to plastic containers with100 mL of 20% sulfuric acid solution (H_2SO_4). After 24 hours of collection, total urine volume was quantified, homogenized and a 10 mL aliquot of each urine sample was gassed and diluted in 40 mL of 0.036 N sulfuric acid (Valadares et al., 1999), to avoid bacterial degradation of purine derivatives (PD) and uric acid precipitation. A composite sample was obtained for the two days of urine sampling for each animal at the end of each experimental period. Samples were stored at -20 °C for further analysis of purine derivatives (uric acid, allantoin, hypoxanthine and xanthine) and urea.

All animals were weighed at the end of the IFP in order to obtain the partial total weight gain, when half of the animals were reversed to another CP contents in diet. The weighing was made at the beginning and at the end of the experimental trial to evaluate

the productive performance, after an application of 16-hours fasting period of solids in all animals.

All animals were slaughtered in a commercial slaughterhouse at the end of the experimental trial, following all procedures necessary to carry out humanitarian slaughter after 16 h of solids fasting. The animals were desensitized by cerebral concussion, followed by complete bleeding through the carotid and jugular section. Carcasses were weighed immediately after slaughter, and after being cooled to - 4 °C for 24 hours, respectively, to obtain the hot carcass weight (HCW) and cold carcass weight (CCW).

After these procedures, sections of *Longissimus dorsi* (LD) muscle between the 12^{th} and 13^{th} ribs were collected from the left half-carcass of each animal to perform measurements of the loin-eye area and the thickness of the subcutaneous fat. A contour of the cranial portion of the muscle was performed on transparency sheets using an appropriate pen, and after scanning the images in computer software the loin-eye area was measured in each half-carcass. The thickness of subcutaneous fat was measured with the aid of a Pachymeter.

4.2. Trial 2 – Ruminal fermentation trial

All of the procedures with the animal's care and handling were approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Sciences of the Federal University of Bahia under protocol number 33-2016.

4.2.1. *Animals, experimental design and diets*

This experiment was conducted at the experimental farm of the School of Veterinary Medicine and Animal Sciences of Federal University of Bahia, located at the municipality of São Gonçalo dos Campos, Bahia State, Brazil. All chemical analyzes

were performed at the Laboratory of Animal Nutrition of the School of Veterinary Medicine and Animal Sciences of the same institution.

Four Santa Ines crossbred sheep, male, castrated, with ruminal fistulas, average body weight (BW) of 60.6 ± 10.6 kg, all at fifteen months of age, were used. The animals were distributed in eight 2 × 2 Latin squares (Figure 2) and were housed in individual metabolic cages with a total area of 1.3 m^2 totally covered, with slatted floor, equipped with individual feeders and water drinkers. The animals were submitted to a 15-d adaptation period, during which they were weighed, identified and dewormed. Experimental period was conducted in four 14-d periods. Each period consisted of eight days for adaptation of the animals to the experimental diets and six days for sampling. All animals were weighed at the beginning and at the end of each experimental period to control weigh variation.

Experimental diets consisted of two increasing contents of CP: 130 and 150 g CP/kg DM. The diets consisted of 50% Tifton hay and 50% concentrate on dry basis. The proportion of the ingredients used in concentrates and in the experimental diets was shown in Table 1. The mineral mix and concentrate was the same used in Experiment 1. Experimental diets were provided *ad libitum* twice a day at 8:00 a.m. and 4:00 p.m. in similar proportions. Intake has been adjusted to keep leftovers at maximum 10 to 20% of the offered daily amount on fresh basis.

4.2.2. *Experimental procedures and sampling*

Samples of the supplied hay and leftovers of each animal were daily sampled during the collection period (from the 9th to the 14th day of each experimental period) and were submitted to partial drying in a forced air oven (55 °C) for 72 hours. After drying, each sample was milled through 1 mm sieve in a knife mill (Wiley mill; TECNAL, São

Paulo, SP, Brazil) and then proportionally composed (based on dry weight), per animal and per period. Hay samples were only composed per period. The concentrate ingredients were sampled from the storage bags exactly in the days of the mixtures thereof. Samples were stored for further laboratory analysis.

From the 9th to the 12th day of each experimental period, fecal samples were collected for the determination of nutrient digestibility. All feces were collected during this three consecutive days (Lazzarini et al., 2016) in every four hours, directly from the collection bags adapted to the animals and later, they were packed in plastic containers with. At the end of each 24 hours fecal samples were weighed, homogenized and a daily sample was submitted to partial drying in a forced air oven (55 °C) for 72 hours. Afterwards the samples were milled with 1 mm sieves with a knife mill (Wiley mill; TECNAL, São Paulo, SP, Brazil) and proportionally composed (based on dry weight), per animal and per period.

From the 13rd to 14th day of each experimental period the animals were submitted to total urine collection for nitrogen balance and microbial CP (MCP) synthesis evaluation. Collecting funnels were connected to polyethylene hoses that carried urine to plastic containers with100 mL of 20% sulfuric acid solution (H_2SO_4). After 24 hours of collection, total urine volume was quantified, homogenized and a 10 mL aliquot of each urine sample was gassed and diluted in 40 mL of 0.036 N sulfuric acid (Valadares et al., 1999), to avoid bacterial degradation of purine derivatives (PD) and uric acid precipitation. A composite sample was obtained for the two days of urine sampling for each animal at the end of each experimental period. Samples were stored at - 20 °C for further analysis of purine derivatives (uric acid, allantoin, hypoxanthine and xanthine) and urea.

From the 13th to the 14th day of each experimental period, samples of ruminal contents were collected immediately before (0 hours), 2, 4, 6 and 8 hours after feeding.

An aliquot of 10 mL of ruminal fluid were preserved to determination of the N-ammoniacal (N-NH_3). This sample was filtered on cheesecloth and added in a plastic tube containing 1 mL of H_2SO_4 (50% v/v) and frozen at -20 ° C for further analysis. Ruminal samples with approximately 8 mL of ruminal fluid were preserved to quantify the volatile fatty acids (VFA). These samples were filtered on cheesecloth and added to centrifuge tubes containing 2 mL of 25% metaphosphoric acid and then stored at –20 ºC for further analysis.

On the 14th day, blood samples were taken immediately before and four hours after feeding by jugular vein puncture using Vacutainer® tubes containing EDTA. These samples were immediately centrifuged at 3500 rpm for 15 minutes, and stored at -20 ° C for further analysis of urea concentrations.

4.3. Chemical analysis

The samples of feeds, leftovers and feces were analyzed for DM and mineral matter (MM) according to official methods 934.01 and 942.05 (AOAC, 2005), respectively. The total N was quantified according to the official method 968.06 (AOAC, 2005), and the constant 6.25 was used to obtain the CP. The residual neutral detergent fiber was corrected for ash and protein (apNDF) content by placing the samples in 100 ml autoclavable flasks, following the proportion of 1 g of sample per 100 ml of detergent (Mertens, 2002) with thermostable α-amylase (Novozymes A/S) and without the addition of sodium sulphite. These samples were autoclaved at 110° C (Barbosa et al., 2015). Washing and filtration procedures for apNDF followed the protocol described by Barbosa et al. (2015). Lignin was measured according to the official method 973.18 (AOAC, 2005) using 72% sulfuric acid. The non-fiber carbohydrates (NFC) were quantified according

to Detmann e Valadares Filho (2010), where NFC = 100 – [(%CP − %ureaCP + %urea) + %apNDF + %EE + %MM].

Urea was quantified in urine samples by enzymatic method in the presence of salicylate and sodium hypochlorite, also with commercial kit for analysis (Enzymatic urea – K047, BIOCLIN, Minas Gerais, Brazil). Allantoin, xanthine, and hypoxanthine were determined according to Chen and Gomes (1992). Uric acid was quantified by enzymatic method in uricase and peroxidase enzymes using commercial kit for analysis (Monoreagent uric acid – K139, BIOCLIN, Minas Gerais, Brazil). The absorbed microbial purines and intestinal flow of microbial N were estimated from the equations proposed by Chen and Gomes (1992) for sheep.

The preparation of LD muscle samples for the determination of fatty acid profile were detailed in Cesar et al. (2016). The fatty acid content in muscle tissue measurement was conducted as described by Hara and Radin (1978). The extracted lipids were hydrolyzed and methylated as described by Christie (1982). The samples transmethylated were quantified with a gas chromatograph (Focus CG - Finnigan) equipped with a flame ionization detector and a 100 m Supelco SP-2560 (Supelco Inc., PA, USA) fused silica capillary column (100 m, 0.25 mm and 0.2 μ m film thickness). The column oven temperature was held at 70 °C for 4 min, then increased to 170 °C at a rate of 13 °C min − 1, and subsequently increased to 250 °C at a rate of 35 °C min − 1, and held at 250 °C for 5 min. The gas fluxes were 1.8 mL min − 1 for carrier gas (He), 45 mL min − 1 for make-up gas (N2), 40 mL min − 1 for hydrogen, and 450 mL min − 1 for synthetic flame gas. One μL sample was analyzed. Injector and detector temperatures were 250 and 300 °C, respectively. The fatty acids were identified by comparison of retention time of methyl esters of the samples with standards of fatty acids butter reference BCR-CRM 164, Anhydrous Milk Fat-Producer (BCR Institute for Materials and Reference

Measurements) and also with commercial standard for 37 fatty acids Supelco TM Component FAME Mix (cat 18919, Supelco, Bellefonte, PA). The fatty acid content was quantified by normalizing the area under the curve of methyl esters using Chromquest 4.1 software (Thermo Electron, Italy). The fatty acid content was expressed as a weight percentage (mg/mg). These analyses were performed at the Animal Nutrition and Growth Laboratory at ESALQ, Piracicaba, São Paulo, Brazil.

The concentrations of N-NH_3 in the ruminal fluid were determined by the colorimetric method described by Chaney and Marbach (1962). For VFA analysis (acetate, butyrate and propionate), ruminal fluid samples were centrifuged (12,000 x g, 10 min, 4 °C), and the supernatants were treated as described by Siegfried et al. (1984). The VFA's were analyzed by HPLC using a Dionex final of 3000 Dual HPLC detector (Dionex Corporation, SUNNYVALE, CA, USA), coupled to a Shodex RI-101 refractive index (IR) maintained at 40øC using a column of Phenomenex Rezex ROA ion exchange, 300 × 7.8 millimeters maintained at 45 ° C. The mobile phase was prepared with 5 mmol/L H_2SO_4 and the flow was 0.7 ml/min. The following organic acids concentrations were used for calibration of the standard curve: acetic (20 mmol/L), propionic (10 mmol/L), and butyric acid (10 mmol/L).

4.4. Statistical Analysis

Data from both experiments regarding intake, digestibility, nitrogen metabolism and microbial protein collected on final phase were analyzed using the MIXED procedure of SAS (version 9.2), with the fixed effects represented by the protein levels in the IFP and FPF, as well as the interaction between them. When collected on initial phase, these data were analyzed only with CP content during the IFP as the fixed effect. For Trial 2, which was designed under Latin Square, random variables related to animal and period

were added to the main models described above. Productive performance and muscle tissue fat acids profile was analyzed under the four feeding schemes as following 130-130, 130-150, 150-130 and 150-150 g CP/kg DM, comprising four experimental diet, using the MIXED procedure from SAS (version 9.2). The statistical model to analyze N-NH_3 also included the time-point of sampling as time-repeated measures into each experimental unit (Littell at al., 1998). For all of the statistical procedures, 0.05 was used as the critical probability level for Type I errors. In case of significant effect for interaction between studied factor in Trial 1, the SLICE procedure was used to compare means.

5. RESULTS

5.1. Trial 1

There was no effect (P> 0.05) of IFP CP contents on intakes of DM (P = 0.59), OM (P = 0.63), digestible OM (dOM, P = 0.99), apNDF (P = 0.48) and NFC (P = 0.18) and on total digestible nutrients (TDN) intake (P = 0.48) (Table 2). As expected, the animals fed with 150 g CP/kg DM in IFP presented higher (P < 0.01) CP intake in relation to the others fed with 130 g CP/kg DM. The relationship between CP and dOM intake was higher (P <0.01) at the 150 g CP/kg DM content when compared to 130 g CP/kg DM. There was no effect (P> 0.05) of dietary CP content in IFP on digestibility of DM and on the other dietary constituents.

There was no effect (P> 0.05) of the interaction between CP content in IFP and FPF for any of the nutrient intake and digestibility variables evaluated (Table 3). The CP contents evaluated in the FPF did not affect the DM intake (P = 0.70), OM intake (P = 0.72), dOM intake (P = 0.72), apNFD intake (P = 0.49) and NFC (P = 0.26). However, CP intake (P = 0.01) was higher for animals fed with 150 g/kg CP than those fed with 130 g CP/kg DM. There was treatment effect (P < 0.01) on the relationship between CP and dOM and CP of the diet, being verified a higher relationship in 150 g CP/kg DM in relation to 130 g CP/kg DM. The digestibility of DM and of the other dietary constituents were not affected (P> 0.05) by CP contents in IFP and FPF.

Excretions of urinary (P = 0.03) N were affected by CP content, with mean values of 150 g CP/kg DM being greater than the 130 g CP/kg DM (Table 4). The N retentions and the relationships between N retained and ingested or absorbed were similar between experimental diet evaluated in IFP. There was no interaction (P > 0.05) between CP contents in IFP and FPF for any of the nitrogen balance variables evaluated. There was no effect (P> 0.05) of IFP CP content on the excretions of the nitrogen compounds and N

retention. However, there was an effect of the FPF CP on the excretions of nitrogen compounds, being verified larger (P = 0.01) urinary N excretion in animals fed 150 g CP/kg DM in relation to animals fed with 130 g CP/kg DM. The FPF CP content did not affect the fecal N (P = 0.72), N retentions (P = 0.17) and the relationships between the N retained and N ingested (P = 0.94) or absorbed (P = 0.31). The MCP synthesis and the microbial efficiency expressed in relation to NDT or dOM intake were not affected (P> 0.05) by CP contents regardless the confinement phases (initial or final).

Animals that received 130 g CP/kg DM and 150 g CP/kg DM both in IFP and FPF presented similar average daily gain (ADG, P = 0.48) and final body weight (FBW, P = 0.65) (Table 5). Similarly, CP content in these two phases did not affect (P = 0.58) the subcutaneous fat thickness of all animals.

In general, the concentration of the muscle tissue fatty acids was not affected (P > 0.05) by CP contents (Table 6). The concentrations of saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) were on average 45.6% and 5.9%, respectively. The mean of PUFA:SFA and n-6/n-3 ratios were 0.13 and 0.12, respectively.

5.2. Trial 2

There was no effect of CP content on intakes of DM (P = 0.67), OM (P = 0.69), dMO (P = 0.60), apNDF (P = 0.61), NFC (P = 0.49) and TDN (P = 0.13) (Table 7). The CP intake was greater (P = 0.03) in animals fed with 150 g/kg CP compared to the other fed with 130 g CP/kg DM. The relationship between CP and dOM intake was greater (P = 0.01) at the 150 g CP/kg DM as expected. The digestibility of DM (P = 0.75), OM (P = 0.61), CP (P = 0.20), apNDF (P = 0.50) and NFC (P = 0.88) did not differ between experimental diet.

There was no interaction (P = 0.75) between CP content and sampling time-point for ruminal N-NH_3 concentrations (Figure 3). The animals fed with 150 g CP/kg DM had greater concentrations of ruminal N-NH_3 (P < 0.01) than those fed with 130 g CP/kg DM. In addition, ruminal N-NH_3 concentrations were larger (P < 0.01) two hours after feeding. There was no interaction (P = 0.69) between CP contents and sampling time-point for blood N-urea (BUN) concentrations (Table 8). Greater concentrations of BUN were observed in animals fed with 150 g CP/kg DM in relation to animals fed 130 g CP/kg DM.

Excretions of urinary N were greater (P = 0.01) in animals fed with 150 g CP/kg DM compared to the other receiving 130 g CP/kg DM (Table 9). However, there was no effect of CP content on N retention (P = 0.74) and relationships between N retained and ingested (P = 0.22) or absorbed (P = 0.09). There was no effect of CP content MCP synthesis (P = 0.98) and on the microbial efficiency expressed in relation to TDN (P = 0.77) or dMO intake (P = 0,90).

There was no interaction between the studied factor for ruminal concentrations of acetic (P = 0.65), propionic (P = 0.36) and butyric acids (P = 0.55). CP contents did not affect acetic acid production (P = 0.95), propionic acid (P = 0.26) and butyric acid (P = 0.93) (Table 10).

6. DISCUSSION

The results from this study showed that increasing CP dietary up to 150 g CP/kg DM for Santa Ines sheep did not improve DM or OM intake. Ours findings was in accordance with that preconize by American system (NRC, 2007), which suggest 130 g CP/kg DM for late maturing sheep with BW 30 kg. It is known that insufficient protein could lead to a ruminal NH_3-N deficiency that would depress microbial fermentation and consequently the DM or OM intake (Allen, 2000; Kiran and Mutsvangwa, 2014). Then, we can infer that 130 g CP/kg DM diets for sheep can ensure adequate N supply for growth of ruminal microorganisms and therefore, these CP level is enough to support optimal ruminal fermentation.

The concentrations of ruminal N-NH_3 was lesser at all times points in sheep fed with 130 g CP/kg DM (Average = 5.6 mg/dL) than those fed with 150 g CP/kg DM (Average = 7.8 mg/dL). Ammonia is an end product of CP breakdown in rumen and the primary N source for majority of ruminal bacteria (Morrison and Mackie, 1996; Patra and Saxena, 2011), therefore, it was expected that 150 g CP/kg DM diets would result in greater concentrations of NH_3-N in comparison to 130 g CP/kg DM diets. But, if there were insufficient amounts of NH_3-N, then would have depression in fiber digestion by ruminal microorganism (Allen, 2000, Rufino et al., 2016), which was not observed in our study.

Considering that a ruminal NH_3-N concentration of 5 mg/dL is required to meet the N requirements of the ruminal microorganisms and support maximal growth (Satter and Slyter, 1974), it seems that N-NH_3 obtained in this study were adequate. Increasing approximately 39% in ruminal N-NH_3 concentrations with 150 g CP/kg DM compared to 130 g CP/kg DM diets did not improve fiber or OM digestibility, and neither microbial

CP synthesis. This confirms that N-NH_3 concentrations obtained with 130 g CP/kg DM CP diets did not limit growth of ruminal microorganisms.

The synchronization between fermentation rate and ammonia releasing can influence microbial efficiency for N production (Owens et al., 2014). Despite the greater relationship between protein and MOd intake obtained for sheep fed with 150 g CP/kg DM diets compared to those fed with 130 g CP/kg DM, increasing in microbial protein synthesis was not observed when CP intake increased. This suggests that microbial protein is mainly influenced by synergy between available energy and nitrogenous compounds in rumen, than total daily amount of these substrates (Kim et al., 1999; Owens et al., 2014).

Ruminal concentrations of VFA have long been used to assess the effect of diet on ruminal fermentation (Hall et al., 2015; Hall, 2013). Because VFA are an end product of ruminal fermentation, it is influence by several factors such as rate of fermentation, rate of feed intake, amount of fermentable substrate, dietary composition and digestibility (Hall et al., 2015; López et al., 2003). Our findings showed that increasing CP probably not modify individual VFA concentrations in the rumen.

Likewise, our results agreed with previous ones with sheep (Utisumi et al., 2013, Kiran and Mutsvangwa, 2010), which probably indicates that dietary CP has no effect on VFA concentrations. We can infer that probably there is no improvement on ruminal fermentation when more than 130 g CP/kg DM is provided for sheep. Additionally, changes in individual VFA concentrations were observed over time of feeding in this study. Considering the similar behavior obtained for ruminal NH_3-N concentrations, probably the ruminal fermentation was optimized two hours post feeding.

Meeting the N requirements of ruminal microorganisms is one strategy to improve N utilization for production and to limit N losses (Koening and Beuchemin, 2013). At

final phase evaluated is this study, N absorption increased when CP content increased, which may justify the lack of effect on fecal N excretion. However during the final phase, urinary N excretions showed an increase of 65% for sheep fed with g CP/kg DM diets compared to 130 g CP/kg DM as a result of greater N intake.

There is no agreement in the literature concerning CP effects on animal performance. Previous studies with different dietary CP content for sheep and goat have shown that increased dietary CP could improve average daily gain (Ahmed et al., 2016; Sharifi et al., 2013). However, other ones with sheep presented no effect of dietary CP content on this variable (Van Emon et al., 2012; Hajji et al., 2015). Additionally, no effects of CP changes at latter portion of feeding period have been reported on animal performance (Amaral et al., 2014; Cole et al., 2006).

The variability observed in literature findings could be justified by several factors such as breed, which results in nutrients intake differences. In the present study, Santa Ines sheep continuously fed with 130 g CP/kg DM or 150 g CP/kg DM as well as those fed in switched scheme had performed similarly. The lack of effect on nutrient intake justified the similar performance among the animals, once voluntary feed intake has been considered the main variable that affects animal performance (Riaz et al., 2013). Our results confirmed that no benefits could be found when more than 130 g CP/kg DM is provided and neither when phase feeding is applied for sheep.

Additionally, the similar performance between all sheep suggests that the lower CP diet provided adequate metabolizable protein for gain. This would be possible because in spite the low supply of dietary N, which reduces CP intake, more endougenous N are recycled into the rumen to sustain microbial synthesis (Batista et al., 2017; Lu et al., 2014), which is considered the main source of metabolizable protein for ruminants. It is

important to emphasize that in situations of N deficiency, energy could be directed for fat synthesis rather than protein synthesis.

Considering that during final phase N retention, N retention:N intake and N retention:N absorbed ratios was similar between experimental diet, it is possible affirms that, in spite lower N intake obtained with 130 g CP/kg DM diets, there was no protein limitation for muscle deposition. This may suggests that sheep fed with 130 g CP/kg DM CP diets presented better N usage for anabolic purpose, resulting in reduction in N urinary excretion. Additionally, the greater blood urea concentrations obtained with 150 g CP/kg DM diets reflected lesser total N utilization by sheep, which is likely a result of an excessive supply of dietary CP (Vasconcelos et al., 2009).

In general, the concentration of the muscle tissue fatty acids was not different between diets. Several factors such as differences in breed, forage to concentrate ratio, and weight at slaughter have been associated with changes in fatty acids of muscle tissue (Bas et al., 2000; Pitroff et al., 2006; Al-Suwaiegh et al., 2015). These factors were ajusted to be the same among diets in this study, with exception for dietary CP. Thus, our results probably indicates that fatty acid profile in muscle tissue could not be influenced by CP content. Additionally, the lack of CP effects on fatty acid concentrations confirms that in spite the lower protein:energy ratio obtained with 130 g CP/kg DM diets, there was no limitation in avaiability of these substrates, resulting in similar subcutaneous fat deposition among animals.

We would emphasize that typically some fatty acids have larger proportion in relation to the total of fatty acids in muscle tissue of sheep. According to Bas et al. (2000), the ingredients used to alter CP content in lamb' diets could influence fatty acid composition of adipose tissues and muscles of lambs. These authors reported that addition of soybean meal as the main protein source brought about a significant raise in C16:0

(22.5% of the total); C18:0 (19.3% of the total) an C18:1 (39.3% of the total) on lamb fat deposit. This is in agreement with our study, which was used soybean meal as the main protein source and showed that C16:0, C18:0 and C18:1-cis 9 represented the most abundant fatty acids in lamb carcass. It is important to point out that C16:0 have been associated with damage in human health, because these fatty acid is known to raise colesterol blood (Al- Suwaiegh et al., 2015; McNeill et al., 2012), therefore the lack of effects of the diets on these fatty acids are desirable.

It is well established that conjugated linoleic acids are produced by the imcomplete biohydrogenation of linoleic acid in rumen, and they are considered beneficial for human health (Daniel et al., 2004; Smith et al., 2016). In addition, C18:2n-6 and 18:3n-3 are considered essential fatty acids for ruminants and represents the major fatty acid in grasses family. Our results showed low concentrations of linoleic and linolenic acids. Bas et al. (2000) reported that lambs receiving diets rich in soybean meal presented lower concentration of linolenic acid than grasses-based diets, which could be justified by the higher linolenic acid content in grasses diets (30-35%) in comparison to soybean meal (7-10%). In general, our results indicates that ingredients used in the present study presented low concentrations of essential fatty acids.

Muscle tissue of sheep showed an average 45.6% and 5.9% of SFA and PUFA, respectively. Studies have been reported in which heavier and fatty carcasses contain higher proportions of SFA in comparison to PUFA (Aurosseau et al., 2004; Abubakr et al., 2015). Then, considering that sheep did not showed differences in carcasses atrubuttes and performance, it was not surprisingly that sheep have shown similar proportions in fatty acids among diets. The higher proportion available of SFA for incorporation into the muscle tisseu have been related to the biohydrogenation of dietary unsaturated fat acids in rumen (Al-Suwaiegh et al., 2015; Wood et al., 2008; Pitroff et al., 2006), which justifies

the lower PUFA concentrations in muscle tissue in comparison the SFA obtained in present study.

The differences obtained in the proportions of SFA and PUFA reflected in a low PUFA:SFA ratio (Average = 0.13). It is known that PUFA:SFA and n-6/n-3 ratios is associated for nutritional values of fat, being important to avoide diseases caused for SFA intake (Aghwan et al., 2014). The British Department of Health (1994) recommends that the PUFA:SFA ratio be greater than 0.45, which is above the mean reported in this study. The ideal n-6/n-3 ratio is defined to be 4:1 or less (Simopoulos, 1999), which is within the mean value (0.12) obtained in this study. These findings lead to supose that other factors besides than dietary protein content should be studied as a way to improve meat quality of sheep.

7. CONCLUSION

The present study show that despite the greater N intake obtained for sheep fed with 150 g CP/kg DM, diet with 130 g CP/kg DM is enough to ensure adequate N supply for microbial production and optimize the ruminal fermentation. Additionally, we can infer that 130 g CP/kg DM diet improves N utilization for production and limits N excretion for Santa Ines sheep. The results suggest that no benefits could be found when more than 130 g CP/kg DM is provided and neither when phase feeding is applied for Santa Ines sheep.

Our results demonstrated that the CP content does not change the concentration of the muscle tissue fatty acids of sheep. However the PUFA concentrations are below the recommended values to obtain an adequate nutritional value for human healthy. Our data raises a basis for future work evaluating effect of dietary CP content associated with another factors, which may improve PUFA deposition in sheep muscle tissue.

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Table 1. Proportion and chemical composition of feed used in experimental diets

Item	CP contents, g/kg of DM			
	130		150	
Proportion of feed, g/kg of DM	Concentrate	Diet	Concentrate	Diet
Tifton hay	-	500	-	500
Corn grain	914.0	457.0	812.0	406.0
Soybean meal	50.0	25.0	152.4	76.2
Urea/ammonia sulphate	22.0	11.0	22.0	11.0
Calcarium	6.2	3.1	6.2	3.1
Dicalcium phosphate	4.0	2.0	4.2	2.1
Sodium chloride	2.6	1.3	2.0	1.0
Mineral mixture	1.2	0.6	1.2	0.6
Chemical composition, g/kg				
DM[1]	842.9	884.7	843.4	884.7
OM[2]	950.1	940.7	944.5	937.9
CP[3]	169.7	132.6	211.0	153.2
NDFap[4]	146.3	415.7	151.9	418.5
Lignin	23.8	37.6	23.7	37.6
NFC[5]	601.6	393.9	578.8	358.3
TDN[6]	-	725.2	-	717.9

[1]DM = Dry matter, g/kg of natural matter; [2]OM = Organic matter; [3]CP = crude protein; [4]NDFap = Neutral detergent fiber corrected to ash and protein; [5]NFC = Non-fiber carbohydrates; [6]TDN(%) = Observed total digestible nutrients.

Table 2. Dry matter, nutrient intake and apparent digestibility for sheep fed with different CP contents in initial phase feeding

Item	CP contents, g/kg of DM		SEM[10]	P-value
	130	150		
Intake, kg/day				
DM[1]	0.71	0.73	0.03	0.59
OM[2]	0.67	0.68	0.05	0.63
dOM[3]	0.49	0.49	0.02	0.99
CP[4]	0.10	0.12	0.003	<0.01
NDFap[5]	0.26	0.27	0.01	0.48
NFC[6]	0.29	0.27	0.01	0.18
TDN[7]	0.53	0.57	0.04	0.48
CP:dOM[8]	0.20	0.24	0.01	<0.01
Intake, g/kg of BW[9]				
DM	30.00	31.68	0.84	0.17
NDFap	11.16	11.81	0.35	0.20
Apparent digestibility, g/kg DM				
DM	719.0	702.9	12.3	0.37
OM	732.1	714.1	12.0	0.30
CP	675.4	690.1	12.5	0.42
NDFap	589.3	589.1	24.1	0.99
NFC	864.1	831.9	13.4	0.11

[1]DM = Dry matter, g/kg of natural matter; [2]OM = Organic matter; [3]dOM = Digestible organic matter; [4]CP = Crude protein; [5]NDFap = Neutral detergent fiber corrected to ash and protein; [6]NFC = Non-fiber carbohydrates; [7]TDN = Total digestible nutrients; [8]CP:dOM = crude protein intake/digestible organic matter intake; [9]g/kg of BW = g/kg of body weight;[10]SEM = standard error of mean.

Table 3. Dry matter, nutrient intake and apparent digestibility of sheep fed with different CP contents in final feeding phase

Item	CP contents, g/kg of DM				SEM[10]	P-value		
	130-130	130-150	150-130	150-150		IPF[11]	FPF[12]	IFPxFPF[13]
Intake, g/kg								
DM[1]	0.91	0.94	0.90	0.92	0.06	0.83	0.70	0.89
OM[2]	0.86	0.89	0.85	0.87	0.06	0.84	0.72	0.89
dOM[3]	0.64	0.66	0.62	0.63	0.04	0.58	0.72	0.89
CP[4]	0.13	0.15	0.13	0.15	0.01	0.90	0.01	0.90
NDFap[5]	0.34	0.36	0.32	0.34	0.02	0.62	0.49	0.89
NFC[6]	0.37	0.34	0.37	0.34	0.02	0.89	0.26	0.93
TDN[7]	0.55	0.53	0.54	0.62	0.09	0.67	0.73	0.61
CP:dOM[8]	0.20	0.23	0.20	0.24	0.001	<0.01	<0.01	0.47
Intake, g/kg of BW[9]								
DM	39.85	40.88	38.34	39.72	2.08	0.53	0.57	0.93
NDFap	14.86	15.39	13.62	14.85	0.89	0.33	0.33	0.70
Apparent digestibility, g/kg DM								
DM	758.1	725.5	714.6	724.8	27.3	0.43	0.69	0.46
OM	760.0	728.4	727.4	727.6	26.3	0.53	0.56	0.57
CP	746.7	754.1	692.5	732.7	29.1	0.21	0.42	0.59
NDFap	646.6	602.2	594.1	668.1	42.5	0.88	0.73	0.20
NFC	863.0	831.6	852.9	780.1	28.6	0.29	0.09	0.49

[1]DM = Dry matter, g/kg of natural matter; [2]OM = Organic matter; [3]dOM = Digestible organic matter; [4]CP = Crude protein; [5]NDFap = Neutral detergent fiber corrected to ash and protein; [6]NFC = Non-fiber carbohydrates; [7]TDN = Total digestible nutrients; [8]CP:dOM = crude protein intake/digestible organic matter intake; [9]g/kg of BW = g/kg of body weight; [10]SEM = standard error of mean; [11]IPF = initial feeding phase; [12]FPF = final phase feeding; [13]IFP x FPF = interaction between CP content in initial and final feeding phase.

Table 4. Nitrogen balance, microbial protein synthesis and microbial efficiency of sheep fed with different CP contents in initial and final feeding phase

Item	CP contents, g/kg of DM		SEM[4]	P-value
	130	150		
N intake , g/day	16.12	20.81	0.98	<0.01
N fecal, g/day	5.12	6.37	0.33	0.02
N urinary, g/day	3.07	5.90	0.85	0.03
N retained, g/day	7.86	8.54	1.09	0.66
N absorbed, g/day	10.93	14.44	0.80	<0.01
Nretained (% of Nintake)	46.39	39.89	4.51	0.32
Nretained (% of Nabsorbed)	68.60	57.32	6.24	0.21
MCP[1]	32.82	36.75	2.62	0.31
MCP/NDT[2]	67.07	62.31	7.27	0.65
MCP/dOM[3]	71.59	66.23	7.88	0.64

	130-130	130-150	150-130	150-150	SEM[4]	IPF[5]	FPF[6]	IPF xFPF[7]
N intake , g/day	24.83	30.15	23.25	29.24	2.13	0.56	0.01	0.88
N fecal, g/day	6.13	6.46	7.07	7.12	0.52	0.14	0.72	0.80
N urinary, g/day	4.52	8.22	4.20	6.17	1.02	0.28	0.01	0.44
N retained, g/day	14.18	15.47	11.97	15.95	1.87	0.65	0.17	0.49
N absorbed, g/day	18.70	23.69	16.18	22.12	2.08	0.33	0.01	0.83
Nretained (% of Nintake)	56.18	51.54	50.25	54.25	4.38	0.72	0.94	0.35
Nretained (% of Nabsorbed)	75.58	65.76	72.78	71.91	5.10	0.74	0.31	0.41
MCP	39.78	41.07	52.10	60.52	12.8	0.24	0.72	0.77
MCP/NDT	60.78	53.65	67.54	64.74	16.87	0.61	0.78	0.91
MCP/dOM	64.30	57.01	71.86	68.45	17.93	0.61	0.78	0.92

[1]MCP = microbial crude protein synthesis; [2]MCP/NDT = microbial crude protein synthesis/ total digestible nutrients; [3]MCP/dOM = microbial crude protein synthesis/ digestible organic matter; [4]SEM = standard error of mean; [5]IPF = initial feeding phase; [6]FPF = final phase feeding; [7] IPF xFPF Interaction between CP content in initial and final feeding phase.

Table 5. Animal performance, loin eye area and subcutaneous fat thickness of sheep fed with different CP contents in final feeding phase

Item	CP contents, g/kg of DM				SEM[8]	P-value
	130-130	130-150	150-130	150-150		
fBW[1], kg	27.20	28.33	27.34	29.27	1.27	0.65
ADG[2], g/day	154.34	176.34	163.39	178.98	12.66	0.48
TBWG[3], kg	10.64	12.16	11.25	12.36	0.89	0.49
HCW[4], kg	11.62	12.14	12.01	12.70	0.56	0.61
CCW[5], kg	11.68	12.30	12.03	12.82	0.57	0.56
LEA[6], cm²	10.25	11.13	11.22	10.48	0.76	0.76
SFT[7], mm	1.73	1.68	1.78	1.88	0.11	0.58

[1]fBW = final body weight; [2] ADG = average daily gain; [3]TBG = total body weight gain; [4]HCW = Hot carcass weight (HCW); [5]CCW = cold carcass weight; [6]LEA = loin eye area; [7]SFT = subcutaneous fat thickness; [8]SEM = standard error of mean.

Table 6. Fatty acid composition (g/100g of total fatty acids) of the *longissimus dorsi* muscle of sheep

Item	CP contents, g/kg of DM				SEM[8]	P-value
	130-130	130-150	150-130	150-150		
C12:0	0.06	0.05	0.06	0.06	0.0049	0.85
C14:0	1.64	1.62	1.63	1.68	0.0929	0.97
C14:1*cis*-9	0.06	0.06	0.06	0.07	0.0066	0.53
C15:0	0.20	0.17	0.20	0.22	0.0206	0.41
C16:0	22.80	22.76	22.25	21.79	0.9649	0.88
C16:1*cis*-9	1.62	1.70	1.71	1.81	0.0500	0.15
C17:0	0.88	0.88	0.80	0.80	0.0487	0.51
C17:1	0.43	0.47	0.47	0.54	0.0333	0.19
C18:0	19.68	19.53	19.02	19.14	0.5400	0.81
C18:1 *trans*-11	0.67	0.68	0.70	0.74	0.1028	0.97
C18:2 *cis*-9, *cis*-12	3.19	3.41	3.99	3.49	0.3306	0.38
C18:2 *cis*-9, *trans*-11	0.21	0.21	0.24	0.26	0.0239	0.39
C18:3 *n*-3	0.21	0.23	0.33	0.25	0.0347	0.10
C18:3 *n*-6	0.01	0.02	0.02	0.02	0.0038	0.24
C20:0	0.08	0.07	0.07	0.06	0.0045	0.30
C20:1	0.11	0.11	0.14	0.11	0.0131	0.25
C20:3 *n*-6	0.03	0.03	0.03	0.03	0.0043	0.53
C20:4 *n*-6	1.18	1.05	1.32	1.09	0.1536	0.61
C20:5 *n*-3	0.19	0.17	0.29	0.19	0.0389	0.12
C22:5	0.32	0.26	0.41	0.32	0.0479	0.19
C22:6 *n*-3	0.06	0.06	0.08	0.07	0.0136	0.49
C24:1	0.10	0.09	0.10	0.09	0.0106	0.79
Σ SFA[1]	47.27	46.50	44.02	43.66	1.3082	0.17
Σ MUFA[2]	47.28	48.02	49.17	50.58	1.2314	0.31
Σ PUFA[3]	5.44	5.46	6.79	5.75	0.6043	0.36
Σ *n*-3[4]	0.80	0.72	1.13	0.82	0.1289	0.14
Σ *n*-6[5]	4.37	4.45	5.29	4.58	0.4723	0.50
n-6/*n*-3[6]	0.11	0.12	0.14	0.12	0.0191	0.73
PUFA/SFA[7]	0.11	0.11	0.15	0.14	0.0166	0.29

[1]Σ SAF = Saturated fatty acid; [2]Σ MUFA: Monounsaturated fatty acid; [3]PUFA: Polyunsaturated fatty acid; [4]Σ *n*-3: Omega-3 fatty acid; [5]Σ n-6: Omega-6; [6]n-6/n-3: Σ n-6/Σ n-3;[7] Polyunsaturated fatty acid/ Saturated fatty acid.

Table 7. Dry matter, nutrient intake and apparent digestibility for sheep fed with different CP contents

Item	CP contents, g/kg of DM		SEM[10]	P-value
	130	150		
Intake, kg/day				
DM[1]	1.25	1.31	0.09	0.67
OM[2]	1.18	1.23	0.09	0.69
dOM[3]	0.84	0.89	0.07	0.60
CP[4]	0.17	0.22	0.01	0.03
NDFap[5]	0.47	0.50	0.03	0.61
NFC[6]	0.50	0.47	0.03	0.49
TDN[7]	0.55	0.65	0.05	0.13
CP:dOM[8]	0.21	0.24	<0.01	0.01
Intake, g/kg of BW[9]				
DM	18.44	19.10	1.44	0.73
NDFap	6.98	7.27	0.56	0.69
Apparent digestibility, g/kg DM				
DM	699.1	692.7	15.0	0.75
OM	711.8	727.8	21.4	0.61
CP	722.8	766.4	21.9	0.20
NDFap	557.8	537.9	23.9	0.50
NFC	846.1	849.2	13.7	0.88

[1]DM = Dry matter, g/kg of natural matter; [2]OM = Organic matter; [3]dOM = Digestible organic matter; [4]CP = Crude protein; [5]NDFap = Neutral detergent fiber corrected to ash and protein; [6]NFC = Non-fiber carbohydrates; [7]TDN = Total digestible nutrients; [8]CP:dOM = crude protein intake/digestible organic matter intake; [9]g/kg of BW = g/kg of body weight;[10]SEM = standard error of mean.

Table 8. Blood N-urea concentrations of sheep fed with different CP contents

Item	Sampling time		P-value		
CP contents, g/kg of DM	0	4	T[1]	ST[2]	TxST[3]
130	17.95	20.35	<0.01	0.62	0.69
150	24.34	26.98			

[1]T = Experimental diet: 130 g of crude protein /kg of dry matter and 150 g of crude protein/kg of dry matter; [2]ST = Sampling time, after the morning feeding; [3] Interaction between CP contents and sampling time-point for blood N-urea concentrations

Table 9. Nitrogen balance, microbial protein synthesis and microbial efficiency of sheep fed with different CP contents

Item	CP contents, g/kg of DM		SEM[4]	P-value
	130	150		
N intake , g/day	24.66	32.79	2.36	0.04
N fecal, g/day	7.45	8.19	0.65	0.44
N urinary, g/day	6.93	13.47	1.58	0.01
N retained, g/day	10.28	11.14	1.77	0.74
N absorbed, g/day	17.21	24.61	2.61	0.08
Nretained (% of Nintake)	40.67	33.03	4.43	0.22
Nretained (% of Nabsorbed)	58.34	44.17	7.22	0.09
MCP[1]	45.61	45.76	7.87	0.98
MCP/NDT[2]	49.52	52.54	7.31	0.77
MCP/dOM[3]	52.46	53.86	8.16	0.90

[1]MCP = microbial crude protein synthesis; [2]MCP/NDT = microbial crude protein synthesis/ total digestible nutrients; [3]MCP/dOM = microbial crude protein synthesis/ digestible organic matter; [4]SEM = standard error of mean.

Table 10. Volatile fatty acids ruminal concentrations of sheep fed with different CP contents

Item	Acetate, *mM*		Propionate, *mM*		Butyrate, *mM*	
	CP contents, g/kg of DM					
Sampling time	130	150	130	150	130	150
0	26.76	31.02	6.33	9.90	4.37	5.20
2	22.74	21.51	8.76	8.63	4.05	2.98
4	21.34	22.33	6.44	5.68	3.04	2.62
6	22.98	23.88	7.21	8.93	4.14	4.19
8	34.35	28.84	9.57	9.49	4.07	4.89
SEM	3.45	3.12	1.63	1.65	0.71	0.74
	P-value					
T[1]	0.95		0.26		0.93	
ST[2]	<0.01		0.05		0.04	
TxST[3]	0.65		0.36		0.55	

[1]T = Experimental diet: 130 g of crude protein /kg of dry matter and 150 g of crude protein/kg of dry matter; [2]ST = Sampling time,after the morning feeding; [3] Interaction between CP contents and sampling time; [4]SEM = Standard error of mean.

	Period 1	Period 2
Treatment 1	130	130
Treatment 2	130	150
Treatment 3	150	130
Treatment 4	150	150

Figure 1. Schematic representation of experimental diet (130 = 130 g of CP/kg DM and 150= 150 g of CP/kg DM) distribution in respective periods in Trial 1.

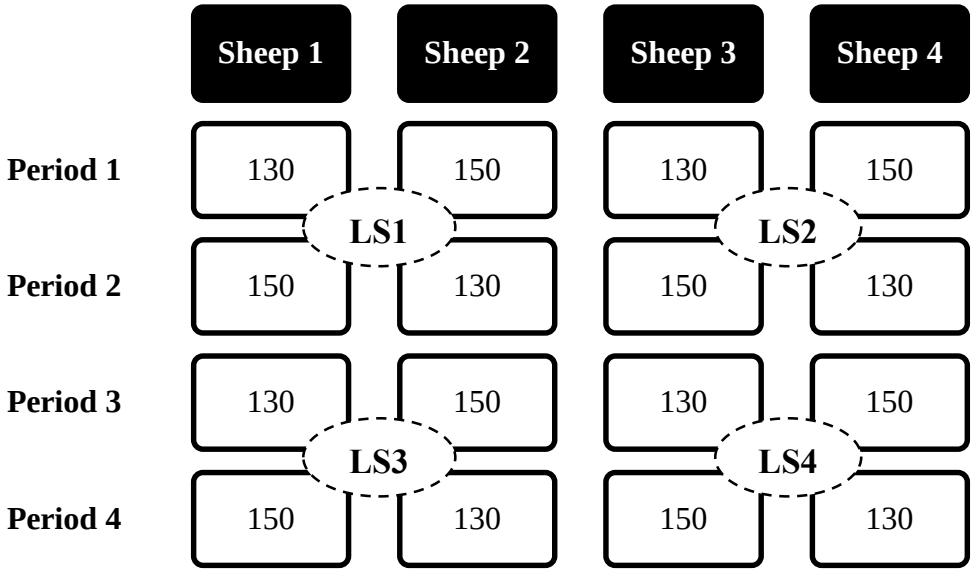

Figure 2. Schematic representation of distribution of animals in eight 2 × 2 Latin squares (LS) in Trial 2.

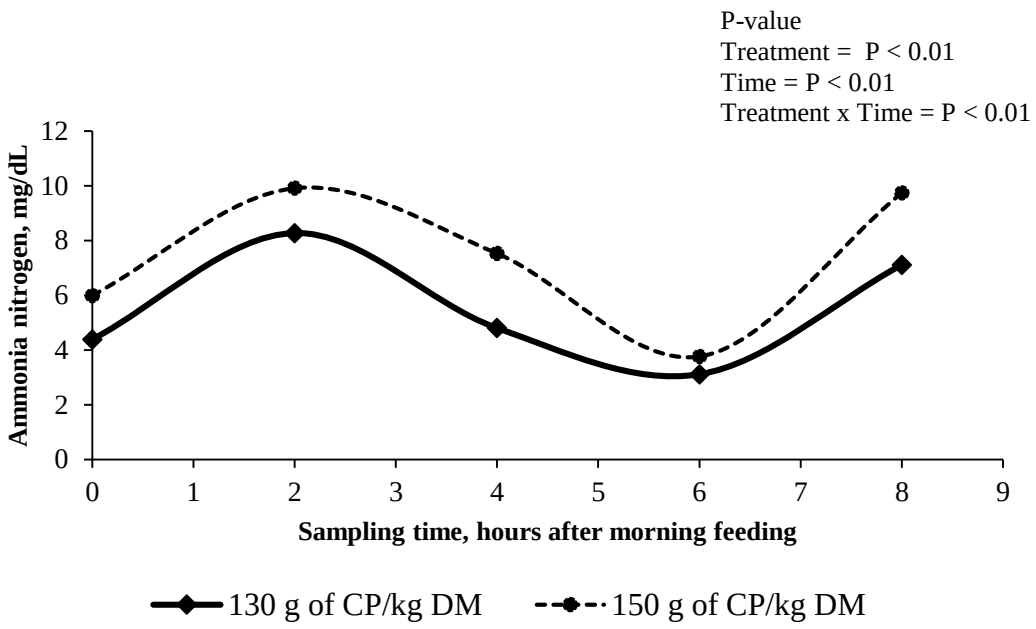

Figure 3. Concentrations of ruminal ammonia nitrogen of sheep at different time points after morning feeding.