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PROGRAMA DE DOUTORADO EM ZOOTECNIA

USO DO LICURI (*Syagrus coronate*) MOIDO NA ALIMENTAÇÃO DE CORDEIROS

ADÍN DAZA GÁRATE

SALVADOR – BA
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GROUND LICURI (*Syagrus coronate*) SEED IN THE FED TO GROWING LAMBS

USO DO LICURI (*Syagrus coronate*) MOIDO NA ALIMENTAÇÃO DE CORDEIROS

Tese apresentada ao Programa de Pós-Graduação em Zootecnia, da Universidade Federal da Bahia, como requisito parcial para a obtenção do título de Doutor em Zootecnia.

Área de Concentração: Produção Animal

Orientador: Dr. Cláudio Vaz Di Mambro Ribeiro

Coorientador: Dr. Thiago Carvalho Da Silva

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DADOS CURRICULARES DO AUTOR

ADÍN DAZA GÁRATE- nasceu na cidade de Aucayacu – Província de Leoncio Prado em 21 de Maio de 1978, filho de Víctor Raúl Daza Torres e Julia Enith Gárate Bardalez. Concluiu o ensino médio em 1993, em 1994 ingressou no curso de Zootecnia da Universidad Nacional Agraria de la Selva (UNAS) – Tingo María. Em dezembro de 1999 concluiu a graduação do curso de Zootecnia. Em abil 2009 inicio o mestrado em Producção Animal na Univerdida Nacional Agraria La Molina (UNALM) – Lima e concluiu em 2001 sob orientação do professor Dr Jorge Vargas Morán.

Em 2012 foi selecionado para o Doutorado em Zootecnia pela Organização dos Estados Americanos (OEA) e o Gurpo Coimbra das Universidades Brasileras (GCUB), com apoio da Divisão de Temas Educativos do Ministerio de Relações Exteriores do Brazil. Em maio de 2013 iniciou o doutorado em Zootenia na Universidade Federal da Bahia (UFBA) e apresentou a defesa de tese na UFBA em Agosto de 2017.

Epígrafe

Faz todo com vontade e amor, que do resto, Deus faz teu caminho e cria tuas oportunidades.

Dedico:

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ABSTRACT

The objectives of this study were to determine the effects the ground licuri in the diets of crossbred Dorper x Santa Inês lambs on its performance and rumen metabolism in two experiments. Experiment 1. The aim was to evaluate intake, apparent digestibility, physical effectiveness NDF, nitrogen balance, microbial protein synthesis, chewing activity, performance, carcass characteristics, and blood parameters of crossbreed x Santa Ines lambs fed increasing levels (0, 5, 10, and 15 % DM basis of the diets) of ground licuri. Forty male lambs (20,89 kg ± 3,97 BW kg) were used in a complete randomized design. The experiment lasted 75 days. The lambs were fed a mixture of Tifton hay (29 %), sisal silage by-products (17 %) and a concentrated mixture (54 %), with 46:54 forage: concentrate rates. The performance was recorded (kg) every 25 days, and the digestibility data were collected between 41 and 45 days. The animals were fed twice daily, at 8:00 h and 16:00 h, ensuring a 10% surplus and water supply was *ad libitum,* and significance was declared at P≤0.05 for both experiments. The licuri inclusion showed decreased linear effects (*P*<0.05) on intake of DM and TDN. The intake and digestibility of EE showed increasing linear effect (*P*<0.05). The apparent digestibility coefficients of DM remained unchanged. The ground licuri inclusion did not affect the nitrogen balance (*P*>0.05). The MPS and EMPS were affected negatively (*P*<0.05). The total weight gain, average daily gain, and carcass characteristics decreased linearly (P<0.05). The ground licuri inclusion did not affect the nitrogen balance (*P*>0.05). The MPS, total weight gain, and carcass characteristics decreased linearly (P<0.05). The chemical, and physical characteristics of *longissimus dorsi* were similar among treatments. The chewing activity was no affected (*P*>0.05) and NDF (g) per bolus decreased linearly (*P*<0.05). The blood urea showed increased linear effect (*P*<0.05), while the serum glucose decreased linearly (*P*<0.05).

Experiment 2. The objective this study was to evaluate the effects of the supplementation of licuri ground 0; 4; 8; 12 and 16% DM basis on the diets on intake, digestibility, nitrogen balance, MPS, EMPS, ruminal parameters, and blood parameters. Five crossbred Dorper x Santa Ines, non-castrated males ram, with average body weight of 42.5 kg were used in a 5 × 5 Latin square design, with 5 periods of 16 days. The lambs

were fed a mixture of tifton hay (26 %), sisal silage (28 %) and a concentrated mixture (47 %) (V:C 53:47). The ground licuri inclusion did not affect (P>0.05) nutrient intake, nutrient digestibility, rumen fermentation parameters, nitrogen balance, MPS, EMPS, and blood parameters. The pH and concentration of NH_3-N, acetate, propionate, butyrate, and total VFA, showed quadratic effect (P<0.05) among collection times (0, 2, 4, 6 and 8 hours after feeding). The ground licuri inclusion showed no effect (P>0.05) on the treatments x time interactions of the rumen fermentation parameters. The ground licuri inclusion increased the NDF, EE, and decreased NFC as was included in the diets.

The animals responses in these experiments were contradictory and therefore required subsequent studies with NDF adjustments in the diet to determine the appropriate level of ground licuri inclusion.

KEY WORDS: performance, VFA, blood parameters, ruminal parameters

1.0 INTRODUCTION

In 2014, the Brazilian sheep herd reached approximately 18 million heads (FAO, 2017), from which 57.5% is located at the northeast region, characterized by the Caatinga biome as the primary forage source with production systems predominantly extensive (IBGE, 2006). The seasonality of fodder production, as well as the need to meet higher animal production, prompt producers in the semi-arid region to seek alternative feed for their herd (SANTOS et al., 2013).

The licuri is native of the Northeaste region of Brazil, being found more than one thousand trees ha^{-1} (SANTOS et al., 2010),and its seed characterized by contained high content of medium chain fatty acids, 60.2 % and 60.5 % of the composition for refined oil and pressed oil, respectively (BAUER et al., 2013), and 10.5% of EE as licuri cake (BORJA et al., 2010). The fats is commonly used for to increase the energy density in the diets of ruminants; and thus, this seed can be used for this purpose.

The licuri has been widely used in research, as licuri cake, to feed sheep (COSTA et al., 2016; NOGUEIRA, 2013; SANTOS, et al., 2015) and goats (BORJA et al., 2014), and as licuri oil to goats (SILVA et al., 2010; LOPES et al., (2010); QUEIROGA et al., 2010; PEREIRA et al., 2010). The supplementation of licuri cake increases the concentration of EE and NDF in the diet and, when fed to lamb up to 24%, led to a 39% reduction on intake, an increase on CP and EE digestibility, and a decrease in total weight gain and average daily gain (COSTA et al., 2016a). However, no effect on intake was observed when licuri cake was included in diets to lambs up to 16% (SANTOS et al., 2015).

The physical-chemical characteristics and the concentration of the NDF is closely related to intake potential (BUXTON, 1996), and variation in dry matter digestibility is related primarily to the concentration and digestibility of NDF (MERTENS, 1969). Licuri fruit may be a nutritional and economically viable alternative to maintain or improve the productivity of feedlot lambs; replacing cereal grains with licuri in the diets of small ruminants may contribute to energy intake while preventing ruminal acidosis. Furthermore, it can help to the value agregation of this vegetable resource. Therefore, this study aims to determine the best level of dietary inclusion of ground licuri on growing

lambs with two experiment; in the first experiment it evaluated the intake, performance and carcass characteristics, and second experiment evaluated the rumen metabolism.

2.0 REVIEW OF LITERATURE

2.1 Sheep production

Sheep farming is present in practically all continents, mainly due to its adaptability to different climates, reliefs, and vegetation. Asia, Africa and Oceania have the world's largest herds, and countries such as Australia and New Zealand control the international meat and sheep-wool market, for their highly technical breeding systems and specialized breeds (VIANA, 2008). Global sheepmeat production for the year 2014 was 14 million tonnes and projected global per capita consumption for the same year was 43.9 kg / year, highest for developed countries (76.1 kg/year) and in developing countries (33.7 kg/year) (FAO, 2016).

In Brazil, sheep farming is destined for both economic exploitation and subsistence of families in rural areas (SIMPLÍCIO, 2001). In 2014, the national sheep herd registered 17,614,454 heads in Brazil, of which 10,126,799 are in the Northeast (57.5%) and 5,166,225 in the Southern Region (29.3%), although their production declined In the years 2011 and 2012, but has a growth trend in the last 10 years (MAGALHÃES et al., 2014); moreover, Brazil has 1.4% of the world's sheep production (SIMPLÍCIO, 2001).

The Northeast of Brazil has most of its territory occupied by xerophilous vegetation, of varied physiognomy and floristic, denominated "caatinga" and occupies about 11% of the national territory, covering the states of Bahia, Sergipe, Alagoas, Pernambuco, Paraíba , Rio Grande do Norte, Ceará, Piauí and Minas Gerais, the caatinga represents about 800,000 km^2, which corresponds to 70% of this region (DRUMOND et al., 2000).

The caatinga is composed mainly of small woody and herbaceous species, usually endowed with spines, and usually deciduous, losing their leaves at the beginning of the dry season are used as fodder (DRUMOND et al., 2000). This region is considered as one

of the most appropriate areas for the exploitation of goats and sheep and in this fact the sheep rationally exploited and conducted in harmony with the economic and social environment is undoubtedly an excellent alternative (SIMPLÍCIO and SIMPLÍCIO, 2006).

2.2 Licuri

The markedly dry seasonality causes need for food and prompts producers in the semi-arid region to seek alternative food for their herds to maintain sheep production (SANTOS et al., 2013). Nevertheless, to the sustainable exploitation of the natural forages of the semi-arid, other native plants can be exploited in the sheep production system and in this region, the licurizeiro (*Syagrus coronata*) is easily found and your fruit (licuri), has a high energy content (CREPALDI et al., 2001); being found more than one thousand trees ha^{-1}, its fruit is fully exploitable, and has been widely explored since colonial times (SANTOS et al., 2010).

The seeds contain the embryon and are surrounded by a distinctive seed coating (BEWLEY and BLACK, 1994). The structures of the seeds such as the endocarp pericarp and the cell walls of the endosperm dictates the extent of digestion (WANG et al., 1999), although after exposure of the endosperm, rumen microorganisms rapidly digest the walls and contents of the endosperm, but their ability to digest the matrix depends on the type of grain or seed (MCALLISTER et al., 2001).

The composition of the kernel of the licuri fruit, from which the oil is extracted, contains around 49.2% of lipids, 11.5% of proteins and 9.7% of total carbohydrates, with a caloric value of 527.3 $kcal.100g^{-1}$ (CREPALDI et al., 2001).

Licuri oil mainly contains crapilic, capric, lauric and myristic fatty acids and, to a lesser extent, palmitic stearic and oleic acid, highlighting myristic and lauric acid (SEGALL et al., 2004). Similarly, Neto et al. (2001) described licuri oil with caprylic (24.68%), capric (13.94%), lauric (36.43%), myristic (7.15%), and palmitic, stearic, oleic and linoleic fatty acids to a lesser extent. Likewise, Bauer et al., (2013), characterized refined oil and pressed oil of licuri with 60.02 and 60.50% of medium chain fatty acids (MCFA) respectively, these fatty acids have 6 to 12 carbons and their composition is very similar to that of coconut oil and have unique properties with important nutritional applications.

The licuri cake has been characterized by Borja et al. (2010) and Nogueira, (2013) as a source of NDF and EE; licuri cake contains CP (19.6%; 15.1%), EE (10.1%; 8.2%), NDF (51.5%; 54.6%), ADF (34.9%; 28.7%) and lignin (17.3%; 16.9%), respectively. Costa et al. (2016), described similar values.

The increased of the concentration of licuri cake in the diets promoted a linear reduction on DM intake (P=0.001), the total weight gain (TWG) and average daily weight gain (ADWG) (P=0.001), N intake, Fecal N, and retained N; furthermore, linearly enhanced (*P*=0.02) the digestibility of CP and EE in lambs; and EE intake showed quadratic effect (COSTA et al., 2016). The inclusion of 50 and 200 g/kg of licuri cake did not showed effect (*P*>0.05) for intake of DM, OM, NDF and TDN; however, increased intake of EE; in addition, the DM and OM digestibility coefficient decreased and, showed remarkable increase of EE digestibility (NOGUEIRA, 2013).

The ADWG presented a quadratic effect (P<0.05) as a function of the increase of licuri cake in the diet and, levels higher than 10.22% may have compromised the animal performance (SANTOS et al., 2015); moreover, for every 8% inclusion, there was an estimated decrease of 0.96 kg in empty body weight.

Likewise, Lopes et al. (2010) included licuri oil in the diet of goats, showed a quadratic effect on DMI and EE digestibility, maximum intake reached 2.3% of oil inclusion, this quadratic effect can be explained by the increase in energy concentration; blood parameters showed no effects.

2.3 Lipids

The lipids are a group of substances found in plant and animal tissues., they are insoluble in water but soluble in common organic solvents such as benzene, ether and chloroform (MCDONALD et al., 2011), plant lipids are of two main types: structural (membranes and protective surface layers) and storage (fruits and seed). Lipids are often classified into the following groups: fatty acids and their derivatives, triacylglycerol's, waxes, phospholipids and sphingolipids (MOTTA, 1980).

The term medium-chain triacylglycerols (MCT) refers to mixed triacylglycerols of saturated fatty acids (FA) with a chain length of 6–10 carbons and sometimes dodecanoic acid (C12:0) and the majority is absorved and transported in the portal vein to the liver, medium chain fatty acids (MCFA) stimulate cholecystokinin secretion, bile

phospholipid and cholesterol secretion less than lengh chain fatty acids LCFAs (MARTEN et al., 2006).

The essentiality of the unsaturated fatty acids, linoleic and linolenic, was described by Burr (1920 to 1930), they are incorporated into membranes as such, and are precursors for other unsaturated FA that are key for metabolic regulation and cell membrane function; linoleic and linolenic acids are precursors for synthesis of the prostaglandins (PALMQUIST, 1964).

Fats are used in ruminant diets, their high caloric density can be useful for overcoming limitations in energy supplies, to manipulate ruminal fermentation, thereby altering digestion and absorption of nutrients (NAGARAJA et al., 1997). The fat and FA metabolism and digestion in ruminants are of considerable interest this interest is based on several reasons, the use of dietary fat supplements by nutritionists to increase the energy density; second, it is now recognized that FA, both of dietary and rumen, can have specific and potent effect on ruminant metabolism and human health; third, we recognize that especific FA produced in the rumen are potente regulators of rumen funtion (OKONKWO et al., 2009). The basic feed ingredients of ruminant diets (forages, cereal grains and supplements) contain about 2-3% fat, and an additional 2-4% may be included as supplemental fat (NAGARAJA et al., 1997). Moreover, generally low negative effects are observed when diets contain less than 50 g added fat/kg (DOREAU and CHILLIARD, 1997).

Source of fat must be carefully considered because fat can negatively affect energy intake and effects of fat on energy intake and partitioning are greater for unsaturated fat sources, although MCFA also can have negative effects (ALLEN, 2011); although feeding fat increases energy density of the diet, it does not ensure that more net energy is available for production (JENKINS and JENNY, 1989).

In the rumen lipids undergo high-intensity metabolism linked to microbial activity, dietary FAs are strongly hydrogenated (CHILLIARD and FERLAY, 2004). The first step in lipid metabolism in the rumen is hydrolysis and the end-products of hydrolysis are free FA such as mono or diglycerides have been found in rumen contents; also, produces glycerol and galactose (DOREAU and CHILLIARD, 1997). The microbial enzymatic action on the components of the food is performed in a short period of time,

the lipase microbial causing the release of constituent fatty acids and *Anaerovibrio lipolytica* is best known (JENKINS, 1993).

Properties of lipids that determine their antimicrobial effects in the rumen include type of functional group, degree of unsaturation, formation of carboxylate salts, and physical association of lipids with surfaces of feed particles and mcrobes (JENKINS, 1993). Moreover, the LCFA are not degradable in the rumen because the anaerobic condition is not favorable for oxidation FA; thus, net synthesis should occur, causing the amount of FA leaving the rumen to be larger than that ingested (WU et al., 1991).

The incorporation of fat in fattening diet tended to reduce the extent of fermentation in the rumen and direct the fermentation to a larger proportion of propionic acid; from the litterature it appeared also that fat reduced apparent digestibility of dry matter and organic matter and increased digestibility of EE (CLINQUART et al., 1995).

The absorption of LCFA is almost exclusively from the small intestine, although MCFA up to and including myristic can be absorbed anterior to the pylorus (SUTTON, 1985). Short-chain FA, up to 12 carbon atoms, are absorbed by the ruminal wall and carried by the ruminal veins, almost 90% of dietary lipids reach the duodenum as non-esterified saturated FA, except when they are protected (DOREAU and FERLAY, 1994).

The L-carnitine facilitates transport of MCFA and LCFA across the mitochondrial; although, has several other functions such as altering the acetyl-CoA: CoA ratio, transporting MCFA from peroxisomes to mitochondria, and modulating flux of intermediates through pathways associated with fatty acid, glucose, and nitrogen metabolism (WHITE et al., 2002).

Loss of FA from the rumen either by absorption across the ruminal epithelium or by catabolism to VFA or CO_2 its minimal, microbes synthesize fatty acids de novo from carbohydrate precursors; therefore, lipid reaching the duodenum consisted of fatty acids from both dietary and microbial origins (JENKINS, 1993), lipids of postruminal digesta are mainly composed of saturated non-esterified fatty acids NEFA (from dietary and microbial origin; 70%) and small and variable amounts of microbial phospholipids (PL) (10 to 20%); all are adsorbed predominantly on particulate matter (BAUCHART, 1993). Much of the earlier work which indicated that fat affects digestion negatively may not be applicable because of great differences in the nature of diets and fats fed and especially in total feed intake (PALMQUIST and JENKINS, 1980).

Fat has negative effects on ruminal activity, mainly on carbohydrate degradation; the incorporation of lipids into fattening diets is highly dependent on the species; in ruminants, fat supplementation is less practised for meat animals than for dairy animals (DOREAU and CHILLIARD, 1997). Palmquist and Conrad (1978), utilized different concentrations of EE in the diets, showed increasing of triglycerides and NEFA; the increased NEFA are probably secondary to hydrolysis of increased blood triglycerides; however, there was no effect on glucose.

In general, the effects of fat supplementation seem to depend on the amount and type of fat; the chain length and degree of esterification and unsaturation of fatty acids (NAGARAJA et al., 1997). Factors that limit utilization of large amounts of fat by ruminants include inhibitory affects on ruminal fermentation, lower intestinal apsortion at high intake, low contribution to total oxidation of nutrients, and sensitivity to nutrient imbalance, causing reduced energy intake (PALMQUIST and JENKINS, 1980).

The inclusion of myristic acid in the diet had no effect on the digestibility coefficient of total DM or CP, but it decreased the digestibility coefficients of crude fibre and nitrogen-free-extractives and increased the digestibility coefficients of fat and minerals (STEELE, 1968). The lauric acid (LA) and coconut oil (CO) decreased protozoal counts by 80% or more compared with control diets (240 g of stearic acid/d), 240 g of LA/d and 530 g of CO/d, decreased ruminal ammonia concentration, moreover, the production of methane decreased with the three diets, the decrease was greater with coconut (HRISTOV et al., 2009). LA consumed at 164 and 243 g/d in the TMR reduced the protozoal population by only 25% and 30% ($P = 0.05$), respectively, showing that these levels, when added to the TMR, were not sufficient to achieve a concentration within the rumen that promoted the antiprotozoal effect of LA (FACIOLA and BRODERICK, 2014).

In general, the methane-suppressing effect of MCFA will be greatest with a feeding regime: administering MCFAwith high proportions of C12:0 and C14:0; using concentrate-based diets; adding minerals (capable of forming soaps) according to requirements; and administering the daily supply of MCFA in few portions (MACHMÜLLER, 2006).

Excessive storage of NEFA as triglyceride compromises liver function while oxidation of NEFA might suppress feed intake; satiety caused by hepatic oxidation of

fuels might be a dominant mechanism controlling feed intake, especially in the peripartum period (ALLEN and PIANTONI, 2013)

Liver uptake of NEFA is proportional to their concentration in the blood; excessive hepatic triglyceride concentration is a result of high blood NEFA concentration and compromises liver function, reducing gluconeogenesis and, therefore, restoration of blood glucose and insulin concentrations, further extending the lipolytic state (ALLEN and PIANTONI, 2013).

Gut peptides that potentially contribute to the control of feed intake include ghrelin, cholecystokinin, and the incretin glucagon like peptide 1 (GLP-1), which also may stimulate insulin secretion (ALLEN and PIANTONI, 2013). Moreover, the ghrelin (hunger hormone secreted by abomasal tissues) is the only gut peptide known currently to stimulate feed intake, likely by increasing gastric emptying and passage rate from the rumen (ALLEN and PIANTONI, 2013)**.**

2.4 Fibre

The detergent system of feed analysis is now accepted by the forage industry in most regions of the USA and has largely replaced the crude fiber system of Van Soest, 1994 (BUXTON, 1996). Diets high in neutral detergent fiber (NDF) often limit intake because of physical fill in the gastrointestinal tract, the physical fill might regulate meal patterns by stimulation of tension receptors and mechanoreceptors in the reticulorumen wall (CHOI and ALLEN, 1999). Cellulose and hemicellulose are highly correlated with indices of lignification, hemicellulose appears to have a special relation to lignin (VAN SOEST, 1967). Generally lignin concentration accounts for 40 to 60% of the variation in cell wall digestion (JUNG, 2012), the lignin is the most important factor that limits cell wall digestion (BUXTON, 1996; JUNG, 2012).

Intake is regulated by complex mechanisms that control the initiation and cessation of feeding behaviour, the metabolism and expenditure of nutrients, and the stability of body weight in the animal (MERTENS, 1996). It is established that the animals can select the diet with a particular sensorial profile due to its metabolic effects and can adjust their diet quantitatively and qualitatively to meet their nutrient requirements (FORBES, 2007).

Moreover, the idea of hepatic glucoreceptors has evolved into the theory that food intake is controlled by a signal from the liver to the brain that is stimulated by oxidation of a variety of fuels, were called this the hepatic oxidation theory (HOT) of the control of food intake, when energy intake of ruminants will be increased when energy consumed per unit of ATP generated in the liver over time is maximized (ALLEN et al., 2009). The primary fuels available to ruminants from consumed starch are propionate, acetate, butyrate, and sometimes lactate from fermentation, glucose from digestion in the small intestine, and lactate from glucose metabolism in intestinal tissues (ALLEN, 2000).

Food intake is the most important factor determining animal performance, diets low digestibility are thought to place constraints on intake, which arise from their slow clearance from the rumen and passage through the gastrointestinal tract (ILLIUS et al., 1996). Some theories assume that intake is controlled by the first limiting factor but this is not satisfying on physiological grounds and there is evidence that signals from feedback factors are integrated in an additive manner (FORBES, 2007).

Physical fill limits intake of forages with high NDF concentration when fed to animals with high energy demands, this is why, NDF concentration is closely related to intake potential of a forage (BUXTON, 1996). The optimal NDF concentration in the ration serves as an upper limit for intake or percentage of forage in the diet which meets the energy needs for a specified production target (MERTENS, 1994).

Slow fiber digestion and passage from the rumen can increase ruminal distention and decrease feed intake, but we are unable to predict the relationship between digestion characteristics of NDF and DMI (ALLEN, 2011).

In fed ruminants, the major substrate for hepatic ketogenesis is likely to be butyrate, in ketosis, the animal increasing amounts of NEFA from adipose tissue, LCFA are the important substrates especially if butyrate supply from the gut is diminished because of inappetence (ZAMMIT, 1990).

The major effect of adding concentrate is one of increasing intake rather than increasing digestibility (WALDO and JORGENSEN, 1980). In addition, more digestible fiber might increase the energy density of diets and microbial N production. Moreover, a one-unit increase in forage NDF digestibility in vitro or in situ was associated with a 0.17 kg increase in DMI in cow (OBA and ALLEN, 1999). In addition, marginal increases in NDF digestibility might not be linearly related to animal responses; 1 unit of enhanced

NDF digestibility at 30% (i.e., 31% vs. 30%) probably influences animal performance to greater extent than 1 unit of enhanced NDF digestibility at 60% (i.e., 61% vs. 60%) and forages with high NDF digestibility were associated with greater DMI (OBA and ALLEN, 1999).

Physical constraints are related to the fill caused by the weight or volume of digesta in the reticulo-rumen (RR). The amount of neutral detergent fiber (NDF) in the rumen (RNDF) may be used as an indicator of rumen fill; however, due to the heterogeneous nature of dietary NDF, predictions of RNDF based on NDF intake were not precise (HUHTANEN et al., 2016).

Mertens and Ely, (1979), proposed a relatively simple dynamic model describing the disappearance of fiber from the digestive tract of a ruminant animal, the model demonstrated that a 1.0% decrease in digestion rates results in a 0.6% increase in maximum digestible dry matter intake, a 1.0% decrease in turnover time results in a 0.9% increase in maximum digestible dry matter intake, and a 1.0% decrease in the indigestible fraction results in a 1.0% increase in maximum digestible dry matter intake.

Mertens, (1987, 1994) developed the NDF-Energy intake system, this approach is based on a theoretical relationship between dietary NDF and energy (i.e., intake is limited by the energy demand of the animal or the physical fill of the diet).

Huhtanen et al., (2007) in evaluating the factors affecting silage dry matter intake (SDMI) of dairy cows, results imply that physical fill is not limiting SDMI of highly digestible grass silages and that both physical and metabolic factors constrain total DM intake in an interactive manner.

Detmann et al., (2014) showed the model where DMI is simultaneously regulated by both physical constraints and metabolic feedbacks, with estimate of variables D-value and B-value. D-value corresponds to the dietary content of digested OM and is associated with energy content of the diet and B-value is defined as the undigested fraction of NDF and indicates bulkiness of the diet, where DMI presented significant assoiaction com both D-value and B-value, DMI responsed quadrtically to the D-value (P=0.042) and the DMI descreased linearly as B-value of the diet increased (P=0.042).

In the same way Huhtanen et al., (2016), of 84 studies develop equations predicting of neutral detergent fiber in the rumen (RNDF) from diet and animal characteristics using a meta-analysis technique, showed that the predictions were

markedly improved by dividing NDF into potentially digestible and indigestible fractions, because rumen turnover time of indigestible NDF was 2.7 times longer than that of potentially digestible NDF. At equal NDF intake, RNDF was negatively associated with dietary crude protein concentration and positively with the proportion of concentrate in the diet. Models based on fecal NDF output generally performed better than those based on NDF intake.

The end-products of microbial fermentation are volatile fatty acids (mainly acetic, propionic, and butyric acids), carbon dioxide, methane, and ammonia. Energy (as ATP) is conserved during fermentation mainly by substrate linked phosphorylation and to a limited extent by electron transport linked phosphorylation, particularly in propionate and succinate producing bactéria (NAGARAJA et al., 1997).

Volatile fatty acids (VFA) and physical fill are considered satiety factors in ruminants; however, in studies where VFA were infused as their salts, depression of feed intake may have been from increased ruminal osmolality rather than specific effects of VFA (CHOI and ALLEN, 1999). Degree of stimulation of ruminal epithelial receptors by VFA and possibly electrolytes, and hepatic receptors by propionate is determined by the rate and extent of fermentation of feeds in the RR. (ALLEN, 2000).

Butyrate in rumen fluid is largely converted to β-OH butyrate as it is absorbed through the rumen wall and is quantitatively less important than either acetate or propionate and the liver removes a large proportion of the propionate and prevents it entering the general circulation; moreover, propionate is more hypophagic than acetate or butyrate when infused into the portal vein of sheep (ANIL and FORBES, 1980).

Propionate concentrations as low as 0.8 mM produced 50% of the maximal propionate induced inhibition of ketogenesis by isolated sheep hepatocytes, suggesting that propionate could be physiologically important for the regulation of LCFA oxidation (JESSE et al., 1986). This inhibition may occur because propionate is converted to methylmalonyl CoA, a molecule that can inhibit carnitine acyltransferase-1 (CAT1), which is necessary for the transport of NEFA into the mitochondria; methylmalonyl- CoA is an intermediate of propionate metabolism (ALLEN and PIANTONI, 2013). The methylmalonyl-CoA can be formed through carboxylation of propionyl-CoA either in the mitochondrial matrix or in the cytosol through the action of propionyl-CoA carboxylase and acetyl-CoA carboxylase (BRINDLE et al., 1985).

Ruminal protein degradation is affected by pH and the predominant species of microbial population, ruminal proteolytic activity decreases as pH decreases with high-forage dairy cattle-type rations, but not in high-concentrate beef-type rations (LENG and NOLAN, 1984).

Increased amounts of fiber in dairy rations stimulate chewing activity and reduce acid production and the cascade of events leading to a decrease in animal performance when too little effective fiber is fed includes decreased chewing activity, leading to less salivary buffer secretion (MERTENS, 1996)

The concept of physically effective NDF (peNDF) has been proposed to estimate the NDF portion of the diet that stimulates chewing activity and possibly the formation of the rumen mat (MERTENS, 1997). A framework for routine measurement of ration peNDF concentration has also been proposed by Mertens (1997).

Mertens (1997), has proposed that this factor may be estimated by measuring the proportion of dry matter retained on a 1.18-mm sieve after the sieve is vertical shaken and multiplying this by the NDF content; the 1.18-mm sieve was chosen because particles > 1.18-mm are believed to be resistant to passage out of the rumen.

Mertens (1997) has suggested that a TMR should contain a minimum of 22 % peNDF to adequately stimulate the amount of chewing activity required to maintain an average rumen pH of greater than 6.0.

Recently developed models that synthesize the effects of both peNDF and fermentable starch on rumen metabolism appear to provide an appropriate basis for estimation of dietary fiber adequacy, the data suggest that a period lasting more than 5 to 6 h/d during which ruminal pH is <5.8 should be avoided to minimize health disturbances due to subacute rumen acidosis SARA (ZEBELI et al., 2012).

Modeling approaches recommends that average amounts of 31.2% peNDF inclusive particles >1.18 mm (i.e., peNDF>1.18) or 18.5% peNDF inclusive particles >8 mm (i.e., peNDF>8) in the diet (DM basis) are required; however, the inclusion of a concentration of peNDF>8 in the diet beyond 14.9% of diet DM may lower DM intake level. (ZEBELI et al., 2012).

Physically effective NDF is defined specifically as the fraction of fiber that stimulates chewing and contributes to the floating mat of large particles in the rumen (MERTENS, 1997). The Penn State Particle Separator (PSPS) is a quick and cost-

effective method to estimate particle size of forage and TMR, and is widely used on- farm to evaluate peNDF (LAMMERS et al., 1996). Physically effective NDF (peNDF) is a measure that reflects the ability of physical characteristics of fiber, mainly particle size, to stimulate chewing and saliva buffering in the rumen (MERTENS, 1997).

The concept of peNDF was proposed to be a measure that was more restrictive than effective NDF and would accurately predict the cow's chewing response to forage/feed particle size. The peNDF of a feed is the product of the NDF content multiplied by a physical effectiveness factor (pef), the pef ranges between 0 and 1 (not effective to 100% effective at stimulating chewing) (GRANT et al., 2005).

Acetic acid (mostly acetate at rumen pH) is produced primarily from fermentation of fibre and is the most abundant SCFA and propionate is produced primarily from fermentation of starch and its rate of production and absorption is much greater than acetate because starch ferments faster than fibre and propionate is absorbed more quickly than acetate (ALLEN and BRADFORD, 2012) lipid supplementation generally resulted in either a decrease or, more often, no variation in ammonia concentration in the rumen, however, very few experiments show an increase in ammonia concentration (DOREAU and FERLAY, 1995).

The excessive excreted N is an important environmental concern, and improving N utilization is a major challenge in ruminant nutrition research (FACIOLA and BRODERICK, 2014). In the same study, dietary tallow and Ca salts of fatty acids decreased both duodenal flow and net small intestinal disappearance of total AA N as a result of a slight reduction in DMI and a decrease in microbial protein synthesis when fat was substituted for fermentable starch in the diet fed (REYNOLDS et al., 1994).

Many bacteria and all protozoa participate in rumen degradation of protein by synthesizing and using a variety of proteases, peptidases and deaminases, the protozoa and anaerobic fungi are also involved in protein breakdown but are less active than bacteria (HRISTOV and JOUANY, 2005).

Several strategies can be used to increase the conversion of feed N into meat and milk protein and to reduce N wastage; first, to feed for increased synthesis of microbial protein, finetune and balance the supply of rumen-degraded feed protein (RDP) and rumen-undegraded feed protein (RUP) and third, balance diets more precisely for essential AA (EAA) (HRISTOV and JOUANY, 2005).

In lambs, the absence of protozoarios increased availability of nutrients supplying both energy and aminoacids since the efficiency of food utilization and the rate of wool growth were increased on the low level of protein supplementation without apparently increasing food intake (BIRD et al., 1979).

Holotrich protozoa had very little effect on the protein metabolism in the rumen, but cellulolytic protozoa (*Polyplastron multivesiculatum, Epidinium ecaudatum, and Eudiplodinium maggi*) and *Entodinium caudatum* decreased the efficiency of protein utilization by the ruminant host (IVAN et al., 2000)

High rates of bacterial protein turnover occurred only in the presence of ciliate protozoa, the rate of bacterial protein breakdown in the absence of protozoa was much less, the influence on the rate at which bacterial protein breaks down in rumen fluid (WALLACE and MCPHERSON, 1987).

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CHAPTER I

Effect of inclusion of ground licuri in fattening diets on intake, digestibility, performance and carcasses characteristics of lambs

Effect of inclusion of ground licuri in fattening diets on intake, digestibility, performance and carcasses characteristics of lambs

ABSTRACT

This study was performed to evaluate intake, apparent digestibility, physical effectiveness NDF, nitrogen balance, microbial protein synthesis, chewing activity, performance, carcass characteristics, and blood parameters of crossbreed Dorper x Santa Ines lambs fed increasing levels of ground licuri. Forty male lambs (20,89 kg ± 3,97 BW kg) were used in a complete randomized design. The animals were housed in feedlot cage, fed ad libitum twice daily, at 0800 and 1600 h. The experiment lasted 75 days. For the treatments, ground licuri was included at levels of 0, 5, 10, and 15 % basis of DM of the diets in substitution of corn. The lambs were fed a mixture of Tifton hay (29 %), sisal silage by-products (17 %) and a concentrated mixture (54 %), with 46:54 forage: concentrate rates. The physical effectiveness NDF and chewing activity were realized on 66 to 67 days. The performance was recorded (kg) every 25 days, previous fast and the digestibility data were collected between days 41 and 45. Significance was declared at $P \leq 0.05$. The licuri inclusion markedly increased the NDF, peNDF, and EE of the diets. The licuri inclusion showed decreased linear effects ($P<0.05$) on intake of DM, CP, NDF, NFC and TDN. However, the intake and digestibility of EE showed increasing linear effect ($P<0.05$), whereas the apparent digestibility coefficients of DM, CP, and NDF remained unchanged. The licuri inclusion did not affect the nitrogen balance ($P>0.05$), although the N fecal excretion had a linear decreased ($P<0.05$). The microbial protein synthesis and efficiency microbial protein synthesis were affected negatively ($P<0.05$). The total weight gain, average daily gain, and carcass characteristics decreased linearly ($P<0.05$) as ground licuri was included in the diets. The feed conversion was not affected ($P>0.05$). Likewise, chemical, and physical characteristics of *Longissimus dorsi* were similar among treatments ($P>0.05$). Despite of an increase in peNDF as licuri was included in the diets, the chewing activity was no affected ($P>0.05$). In addition, the licuri inclusion had no effect ($P>0.05$) on the serum concentration of creatinine, albumin, NEFA, TG, ALT, and AST; nevertheless, the blood urea showed increased linear effect

(P<0.05), while the serum glucose decreased linearly (P<0.05). The licuri inclusion decreased DMI by physical fill effect to the NDF in the reticulorumen, and thus negatively affected the microbial protein synthesis, lambs performance, and carcass characteristics. However, the licuri did not affect the physical and chemical characteristics of the *longissimus dorsi* muscle.

Abbreviations: DM, dry matter; DMI, dry matter intake; CP, crude protein; NDF, neutral detergent fiber; peNDF, physically effectiveness NDF; NFC, non-fibrous carbohydrates; ADG average daily gain; NEFA Non-esterified fatty acids; TG Triglyceride; MPS, microbial protein synthesis; EMPS efficiency microbial protein synthesis

Key words: byproduct, intake; digestibility, *longissimus dorsi*, performance

1.0 INTRODUCTION

The corn is an energy ingredient widely used in the diet of feedlot lambs and represents a large proportion of the cost of feeding. Therefore, the evaluation of lower cost alternative feed to replace traditional feed is of great importance in maintaining or improving the profitability of sheep production. The licuri (*Syagrus coronata*), a native palm tree of the northeast of Brazil, its can be found at farms of sheep producers in semiarid regions, its fruit is a drupe with abundant endosperm, an oily, fibrous mesocarp and the seeds when dry, are of dark color and of hard integument (endocarp) that covers the kernel rich in oil that contains around 60.0 % medium chain fatty acids (BAUER et al., 2013).

The licuri seed is an important food resource for humans and animals in the northeast Brazil, although in the animal production the licuri has been widely fed as licuri cake that is characterized to increase the concentration of EE and NDF in the diet to sheep (COSTA et al., 2016; SANTOS et al., 2015) and goats (BORJA et al., 2014). The inclusion licuri cake when fed to lambs up to 24%, led to a 39 % reduction on intake, increased CP and EE digestibility, and decreased on total weight gain and average daily gain (COSTA et al., 2016) but, nevertheless, Santos et al. (2015) reported no effect on intake was observed when up to 16% licuri cake was included in diets to lambs. Likewise, also the licuri oil has been used in some studies in goats (SILVA et al., 2010; LOPES et al., 2010; QUEIROGA et al., 2010; PEREIRA et al., 2010); however, no studies have been reported study using ground licuri seed plus endocarp in the feeding of lambs.

The aim of this study was to determine the best level of dietary inclusion of ground licuri in substitution of corn on intake, performance and carcasses characteristics of lambs.

2.0 MATERIAL AND METHODS

2.1 Protocol, animals and general procedures

All the procedures and protocols used in this experimental study were approved by the Committee of Ethics in the Use of Animals at the Federal University of Bahia (CEUA-UFBA). The study was carried out in the farm São Gonçalo dos Campos - UFBA, Bahia, Brazil. Forty, non-castred, crossbred Dorper x Santa Ines lambs, with 20.89 kg (± 3.97) of average BW were used in a complete randomized design. The experiment lasted 75 days, plus 10 days of adaptation. External and internal parasites were controlled with ivermectin and vaccinated against *Clostridium sp* during the adaptation period. The animals were housed individualy in feedlot cage (1.00 x 1.00 m).

2.2 Ingredients, diets, and chemical composition

The licuri seeds with their endocarp (will be called ground licuri) were ground in a grass chopper machine (TFC150 MIN 500, Incomagri, Brazil) with a 2.0 mm sieve before mixed in the concentrate, it will be called ground licuri. The treatment diets consisted of levels of ground licuri inclusion (DM basis) to the diets as follow: 1) 0% ; 2) 5%; 3) 10%; and 4) 15% of ground licuri in the total diet. All experimentals diets were formulated to meet the requirements for 200 g.day^{-1} of weight gain according to NRC (2007).

The diets were composed of tifton (*Cynodon spp.*) hay, sisal (*Agave sisalana*) silage and concentrate in the proportions 29, 17 and 54%, recpectively (46:54 of forage:concentrate ratio); tables 1 and 2.

2.3 Intake and digestibility

The lambs were fed twice daily, at 8:00 h and 16:00 h, ensuring a 10% surplus and water supply was *ad libitum*. Individual intakes were recorded daily. 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. The non-fiber carbohydrates (NFC) were calculated according to Hall (2000).

NFC = 100 - [(% CP - % CP urea + % urea) + % EE + % ash + % NDF)]

In which: CP= Crude protein; EE = Ether extract; NDF = neutral detergent fiber.

The TDN values were estimated for the different diets by the equation:

TDN = DCP + (DEE × 2.25) + DNDF + DNFC (SNIFFEN et al., 1992)

In which DCP = digestible CP; DEE = digestible EE; DNDF = digestible NDF; and DNFC = digestible NFC.

The TDN intake (TDNi) was calculated, according to Sniffen et al. (1992) using the equation:

$TDNi\ (g.day^{-1}) = (CPi - CPf) + (2.25\ (EEi - EEf)) + TCi - TCf$

In which: CPi, EEi, and TCi represent the intake of CP, EE, and total carbohydrates, respectively and where CPf, EEf, and TCf refer to the excretion of CP, EE, and total carbohydrate in the feces, respectively.

Table 1.Nutrient composition, particle distribution, physical effectiveness factor (pef), and physically effective fiber (peNDF) of dietary ingredients

Item	Ingredients				
	Tifton hay	Sisal silage by-products	Ground corn	Soybean meal	Ground licuri
Nutrients[1]					
DM ($g.kg^{-1}$ fresh weight)	907	120	872	874	914
Organic matter	939	908	989	936	976
Mineral matter	61	92	11	64	24
Crude protein	62	67	97	518	60
Ether extract	11	13	31	26	91
Neutral detergent fiber	738	274	113	177	672
Acid detergent fiber	350	188	22	59	393
Cellulose	249	176	29	66	188
Hemicellulose	316	110	56	34	237
Lignin	44	93	22	22	165
Non-fibrous carbohydrates	128	554	766	226	155
Particle size distribution[2]					
19 mm	67.56	5.94	0.00	0.00	0.00
8 mm	11.71	9.54	0.00	0.00	29.18
1.18 mm	13.71	65.52	54.31	56.08	61.97
Pan	7.02	19.00	45.69	43.92	8.85
$pef_{8.0}$	0.79	0.15	0.00	0.00	0.29
$pef_{1.18}$	0.93	0.81	0.54	0.56	0.91
$peNDF_{8.0}$, % of DM	58.32	4.11	0.00	0.00	19.50
$peNDF_{1.18}$, % of DM	68.61	22.21	6.11	9.93	61.19

[1]Expressed as $g.kg^{-1}$ DM. [2]Particle size distribution of ingredients was measured using the Penn State Particle Separator (Kononoff et al., 2003); $pef_{8.0}$ and $pef_{1.18}$ = physical effectiveness factor determined as the proportion of particles retained on 2 sieves (Lammers et al., 1996) and on 3 sieves (Kononoff et al., 2003), respectively; $peNDF_{8.0}$ and $peNDF_{1.18}$ = physically effective NDF determined as NDF content of the diet multiplied by $pef_{8.0}$ and $pef_{1.18}$, respectively.

The TDN content was calculated by the equation Sniffen et al. (1992):

TDN = intake of TDN/intake of DM

The chemical composition of the consumed feed was estimated using the ratio of the intake of each nutrient to the intake of DM × 100. Between days 41 and 45, daily

Table 2. Ingredients, nutrient composition, particle distribution, physical effectiveness factor (pef), and physically effective fiber (peNDF) contents of experimental diets

Item	Treatments[1]			
	0	5	10	15
Ingredients[2]				
Ground Licuri	0	56	112	169
Vitamin/mineral mix	11	11	11	11
Soybean meal	142	145	146	149
Ground corn	376	321	263	208
Urea/ammonium sulphate	11	11	11	11
Tifton hay	290	290	289	288
Sisal silage by-products	167	167	167	165
Nutrients[2]				
Dry matter ($g.kg^{-1}$ fresh weight)	757	762	764	768
Organic matter	942	942	941	940
Mineral matter	58	58	59	60
Crude protein	168	168	166	165
Ether extract	21	24	28	31
Neutral detergent fibre	328	359	390	421
Acid detergent fibre	150	170	191	211
Cellulose	122	131	140	148
Hemicellulose	136	146	156	166
Lignin	40	48	56	64
Non-fibrous carbohydrates	451	417	382	347
Particle size distribution[3]				
% of DM retained on sieves				
19.0-mm	20.92	20.90	20.81	20.72
8.0-mm	4.95	6.32	7.66	8.99
1.18-mm	43.10	43.81	44.42	45.01
Pan	31.01	28.91	27.12	23.13
$pef_{8.0}$	0.26	0.27	0.28	0.30
$pef_{1.18}$	0.69	0.71	0.73	0.75
$peNDF_{8.0}$, % of DM	8.50	9.79	11.12	12.50
$peNDF_{1.18}$, % of DM	22.66	25.47	28.46	31.45

[1]Percentage inclusion of ground licuri in the diets. [2]Expressed as $g.kg^{-1}$ DM. [3]Particle size distribution of mixed diets was measured using the Penn State Particle Separator (Kononoff et al., 2003); $pef_{8.0}$ and $pef_{1.18}$ = physical effectiveness factor determined as the proportion of particles retained on 2 sieves (Lammers et al., 1996) and on 3 sieves (Kononoff et al., 2003), respectively; $peNDF_{8.0}$ and $peNDF_{1.18}$ = physically effective NDF determined as NDF content of the diet multiplied by $pef_{8.0}$ and $pef_{1.18}$, respectively.

samples of TMR, orts and feces from 20 animals were collected, weighed by each animal to estimate the apparent digestibility coefficient. Feces were collected by the total collection method with the aid of appropriate canvas bags attached to the animals using nylon strips. Samples were mixed and a composite sample was taken, dried at 55 °C and stored for further analysis.

All the samples of ingredients, feed, feces and orts were ground (Moinho Willye TE-680, TECNAL, Brazil) through a 1 mm sieve, and were analyzed according to the AOAC (2010) for dry matter (method 934.01), ash (method 942.05), nitrogen (method 2001.11) and ether extract (EE; method 920.39). The NDF and ADF were analysed using heat stable amylase, according to Van Soest et al., (1991).

The coefficient of apparent digestibility (CD) of DM, OM, CP, EE, NDF, NSC and TDN were calculated using the following equation:

CD = (nutrient intake (g) - nutrient excretion (g)) / nutrient intake (g)

2.4 Physical effectiveness NDF and chewing activity

On day 66th and 67th of the study, approximately 150 to 250 g of orts and TMR were collected for determination of particle size. The particle size of these samples was measured in triplicate using the Penn State Particle Separator (PSPS) equipped with 2 sieves: 19.0 and 8.0-mm (LAMMERS et al., 1996) or 03 sieves: 19.0, 8.0, 1.18-mm and a bottom pan (KONONOFF et al., 2003). The operation of this device was similar to that described by Lammers et al. (1996); the sieve set was shaken horizontally (17 cm) five times in one direction, then rotated one fourth turn, and again shaken five times. This steps were repeated for eight sets of five replications for a total of 40 shakes for each sample.

Physically effective factor (**pef**) values for orts and TMR were determined as the total proportion of DM retained on the 19.0 and 8.0 mm sieves (**pef$_{8.0}$**; (LAMMERS; et al., 1996) or on the 19.0, 8.0 and 1.18-mm sieves (**pef$_{1.18}$**; KONONOFF et al., 2003; Table 2).

The peNDF was calculated by multiplying pef$_{8.0}$ and pef$_{1.18}$ by the NDF content of the diet (DM basis) to obtain peNDF$_{8.0}$ and peNDF$_{1.18}$ (Table 5), respectively (MERTENS, 1997). Likewise, the pef$_{8}$, pef$_{1.18}$, peNDF$_{8.0}$ and peNDF$_{1.18}$ diets consumed were estimated.

Chewing activity was continously monitored and recorded by observation every 5 min for 24 h on day 67th of experimental period. Activity was recorded as eating, ruminating, or idle and each activity was assumed to persist for the entire 5-min interval. The data were used to estimate the eating time, ruminating time, and time spent eating and ruminating per gram of DM and NDF (YANSARI A. T. et al., 2004). Total time spent chewing was calculated as the total time spent eating and ruminating.

On the same day, the ruminating chews per bolus (chewing rate) and time spent per bolus by each lamb in triplicate per each periods of the day (morning, afternoon and night) were recorded. The data collection was performed by four distributed registers so as not to interfere with the animal activities.

2.5 Nitrogen balance and microbial protein synthesis

On the same period of the digestibility (46th day), nitrogen balance were estimated with 20 lambs (five lambs from each treatment), randomly assigned. Urine spot samples (10 ml) were collected with bottles containing 40 ml of 0.036 N H_2SO_4 and then frozen. Total urinary nitrogen were determined by Kjeldahl method (AOAC, 1990) procedure. Nitrogen balance (NB, g.day^{-1}) was calculated as the difference between the total nitrogen intake and the total nitrogen excreted in feces and urine:

The concentrations of creatinine (colorimetric method endpoint - Labtest) and uric acid (colorimetric enzymatic method – Bioclin K139) in the urine were determined. Creatinine was used as a marker to estimate urinary volume, considering that the mean excretion of creatinine per lamb is 23.1 mg creatinine/BW (FAICHNEY et al., 1995). The urinary volume (UV) was estimated by UV (L) = BW * 23.1 mg/kg/creatinine in the urine (mg.L^{-1}).

The urine allantoin, xanthine and hypoxanthine contents were estimated by colorimetric methods and the total purine derivatives (TP) excretion was calculated by the sum of hypoxanthine, xanthine, acid uric and allantoin in the urine and expressed as mmol.day^{-1}. The absorbed purines (AP) were estimated based on the total amount of purine derivatives excreted in sheep (PE_{sheep}), according to Chen and Gomes, (1992):

$$PE_{sheep} = 0.84\ AP + (0.150\ BW^{0.75} * e^{-0.25AP});$$

Where; PE_{sheep}= Purine derivatives excreted in sheep (mmol/d), AP= absorbed purines (mmol.day^{-1}), BW= metabolic body-weight (kg).

The supply of microbial nitrogen (MN) was estimated as follows:

MN (g.day^{-1}) = (70 * AP) / (0.83 * 0.116 * 1000); assuming that the digestibility of microbial purine is 0.83, the N content of purines is 70 mg N.mmol^{-1} and, the purine-N:total-N ratio in mixed rumen microbes is 11.6:100 (CHEN and GOMES, 1992).

2.6 Blood parameters

Blood samples were taken by external jugular vein using beveled needle and 10 ml tubes containing EDTA (ethylene diaminotetraacetic acid). The sample tubes were centrifuged at 3500 rpm for 15 minutes and the serums were placed in Eppendorf tubes and stored at -20 ^{o}C until analysis. Blood samples were collected at 0, 3, 6 and 9 h after feeding on day 45 of the experimental period. Blood triglycerides (enzymatic-Thinder method- Labtest, ref. 87), urea (urease endpoint method - Labtest, ref. 27), glucose (kinetic method- Labtest, ref 133), albumin (Bromocresol Green Method- Labtest, ref. 19), creatinine (colorimetric method endpoint - Kit Labtest, ref 35), non-esterified fatty acids (NEFA; colorimetric method- RANDOX), aspartate aminotransferase (AST; kinetic method UV - Doles) and alanine aminotransferase (ALT; kinetic method UV - Doles) were analized by spectrophotometer (Bio-Rad Laboratories, Hercules, USA) at length specified in each procedures.

2.7 Performance, slaughter procedure, and storage of meat samples

On day 0, 25 and 75 of the experimental period the animals were subjected to a solid fasting for 16 hours, weighed to determine body weight (BW), average body weight (ABW), weight gain (WG), average daily weight gain (ADWG) and, body weight at slaughter (BWS; final BW). The feed conversion ratio (grams feed per grams weight gain) were calculated for each individual lamb.

At the end the experimental period (75 day), lambs were weighed and transported to the comercial slaughterhouse Baby Bode where they were housed by 24 hours in fasting. After were stunned and slaughtered by the sectioning of the jugular vein and the

carotid artery, according to the humanitarian slaughter procedures recommended by the Ministry of Agriculture (MAPA BRASIL, 2000). The carcasses were cut between the 12th and 13th ribs for measuring the fat thickness (Wood and Macfie 1980) with a vernier calliper (Mitutoyo 500-752-10), according to Hopkins et al. (1992). Then, The *Longissimus dorsi* muscles were removed from the right and left carcass sides and frozen for further analysis. At the end of the storage time, the *Longissimus dorsi* muscle was thawed at 4 ° C for 24 hours and the epimysium removed prior to the physical and chemical analyses:

2.8 Proximate chemical analysis of meat

The chemical composition of the meat was determined on the ground *Longissimus dorsi* samples, which were analyzed for dry matter (AOAC, 1990; method 950.46), ash (AOAC, 1990; method 920.153), crude protein (AOAC, 1990; method 981.10) and total lipids (AOAC, 1990; method 960.39).

2.9 Physical analysis of meat

For meat color (*Longissimus dorsi*) measurements a lengthwise cut was performed to expose the meat to the atmosphere for oxygenation of the myoglobin, and after 30 minutes three color measurements were performed in three different positions. The measurements were made with a colorimeter (Model CR400, Konica Minolta, Japan), using the CIE colour System (Commission Internationale de l'Eclairage) which gives the the values for L* (bright- ness), a* (red–green axis), and b* (yellow–blue axis) and hue angle (H*) and chroma (C*) indices were calculated as H*= arctan (b*/a*), expressed in degrees and $C=[(a^*)^2+(b^*)^2]^{0.5}$. C* is related to the quantity of pigments and high values represent a more vivid colour and denote lack of greyness (MILTENBURG et al., 1992), and H* is the attribute of a colour perception denoted by blue, green, yellow, red, purple, etc (HAJJI et al., 2016).

The Water-holding capacity (WHC) was determined on the same day, using the filter paper press method described by Hamm (1986). From each muscle *Longissimus dorsi* sample, a 0.5 g sample of muscle tissue was placed on filter paper and pressed at 10 $kg.min^{-5}$ between two metal plates. Afterward, meat was removed from the paper and the

weight of the meat was recorded (final weight, Wf). The WHC was expressed as percentage of drip loss, calculated as WHC% = (Wi − Wf)/Wi.

For the water cooking loss determination a 6 cm freshly section was trimmed and squared in order to provide an approximately 30 g of rectangular *Longissimus dorsi* sample. The steaks were covered in aluminum foil, cooked in a pre-warmed clam-shell grill (model GR2080B1, George Foreman, China) to an internal temperature of 71 °C in the geometric centre of the steak (measured by Gulterm 700-10S thermometer, Gulton, Brazil). Cooking losses (CL), used as a measure of meat water holding capacity, were determined as the difference in meat sample weights before and after cooking, and expressed as a percentage of initial weights (AMSA, 2015).

The shear force was determined on the steaks that were also used for cooking loss, five round cores (1.27 cm diameter x 2 cm long) were removed from each steak, parallel to the long axis of the muscle fibres. Each core was sheared once through the center, perpendicular to the fiber direction by a Warner-Bratzler shear (WBS) machine (BFG 1000N Basic Force Gauge, Mecmesin, UK), and was compressed by a load cell of 25 kg and a cross-head speed of 2 $mm.s^{-1}$, along 30 mm, were recorded as the average of a minimum of five subsamples, of the maximum force needed to shear force ($kgf.cm^{-2}$) of the samples (AMSA, 2015).

2.10 Statistical Analysis

The data were analyzed using the PROC MIXED of SAS in a completed randomized design. Heterogeneity of variance was tested by the command REPEATED and used when significant. Polinomial contrasts were used to test the linear and quadratic effects of licuri supplementation on all parameters. Initial body weight and blood parameters sampled at day 0 were tested as covariables and used when significant. The temporal effect of the diets on blood parameters after feeding and the weight gain throghout the experimental period was analyzed in a repeated measure design. Before the analyses, the best error structure was defined based on the AIC parameters using the command REPEATED. Degrees of freedom and tests were adjusted using the Kenward-Roger option. The time effect was decomposed in 2 orthogonal polynomial contrasts: linear, quadratic and cubic. Significance was declared at $P < 0.05$.

3.0 RESULTS

3.1 Intake and digestibility

The addition of licuri in the diets markedly increased the content of NDF and the EE, and had an opposite effect on the dietary NFC (Tables 1 and 2). The licuri inclusion showed decreasing linear effects (P<0.05) on intake of DM, OM, PC, NDF, NFC and TDN.

Table 3. Least square means of nutrients intake and digestibility of lambs fed increasing levels of ground licuri

Item	Treatments[1]				SEM[3]	P[2]	
	0	5	10	15		L	Q
Intake (g.day^{-1})							
Dry matter	936	851	864	700	39.01	<0.01	0.32
Organic matter	872	803	807	650	36.31	<0.01	0.24
Crude protein	135	139	138	113	5.83	<0.01	0.01
Ether extract	13	14	27	25	0.84	<0.01	0.19
Neutral detergent Fiber	186	176	158	126	8.30	<0.01	0.20
NFC[4]	313	283	287	208	11.96	<0.01	0.05
TDN[5]	895	825	846	684	37.65	<0.01	0.23
Dry matter (% MBW$^{0.75}$)[6]	11.6	10.1	10.6	8.26	0.59	<0.01	0.47
Digestibility coefficients							
Dry matter	0.654	0.661	0.646	0.614	0.002	0.10	0.30
Organic matter	0.686	0.691	0.675	0.643	0.002	0.06	0.27
Crude protein	0.729	0.739	0.755	0.764	0.002	0.13	0.98
Ether extract	0.743	0.816	0.874	0.836	0.002	<0.01	<0.01
Neutral detergent Fiber	0.529	0.548	0.547	0.491	0.002	0.27	0.11
NFC[4]	0.858	0.849	0.815	0.802	0.001	<0.01	0.83
TDN[5]	0.795	0.789	0.767	0.747	0.001	0.02	0.62

[1]Percentage inclusion of ground licuri. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects. [3]Standard error of the mean. [4]Non-fibrous carbohydrates. [5]Total digestible nutrients [6]Metabolic body weight.

However, the EE intake showed a increasing linear effect (Table 3) and the total digestive digestibility coefficients of DM, OM, CP and NDF were not affected by treatments. In contraste, the inclusion of licuri had increasing linear effect (P<0.05) on digestibility of EE and, showed opposite response in NFC and TDN digestibility.

3.2 Physical effectiveness NDF and chewing activity

In the orts, the proportion of particles retained on the 19.0-mm sieves and **$pef_{8.0}$** value had a decreasing linear effect (P>0.05). In contrast, the proportion of particles retained on the 1.18-mm sieves and $peNDF_{1.18}$ showed a increasing linear effect (P>0.05).

Table 4. Least square means of the particle size distribution, physical effectiveness factor (pef), and physically effective fiber (peNDF) contents in the orts and diets consumed by lambs fed increasing levels of ground licuri

Item	Tratements[1]				SEM[3]	P^2	
	0	5	10	15		L	Q
Orts							
% DM retained on sieve[4]							
19.0-mm	30.31	26.52	4.40	6.10	3.70	<0.01	0.50
8.0-mm	35.41	41.22	35.41	33.20	3.06	0.42	0.21
1.18-mm	22.72	25.11	55.91	58.01	5.11	<0.01	0.98
Pan	1.30	4.76	2.68	2.71	0.93	0.61	0.07
$pef_{8.0}$	0.72	0.71	0.41	0.39	0.06	<0.01	0.94
$pef_{1.18}$	0.99	0.95	0.97	0.97	0.01	0.70	0.07
$peNDF_{8.0}$, % of DM	24.5	35.5	25.7	29.9	5.55	0.79	0.54
$peNDF_{1.18}$, % of DM	32.1	58.7	53.2	79.8	6.01	<0.01	0.99
Diets consumed (adjusted for particle size of orts)							
% DM retained on sieve[4]							
19.0-mm	20.31	20.70	21.72	23.31	0.30	<0.01	0.07
8.0-mm	3.37	3.72	5.37	6.20	0.40	<0.01	0.56
1.18-mm	44.20	45.41	44.02	44.52	0.43	0.76	0.40
Pan	32.11	30.91	28.82	26.31	0.23	<0.01	<0.01
$pef_{8.0}$	0.24	0.24	0.27	0.29	0.01	<0.01	0.13
$pef_{1.18}$	0.68	0.69	0.71	0.74	0.01	<0.01	0.01
$peNDF_{8.0}$, % of DM	9.09	9.22	9.19	11.0	0.26	<0.01	<0.01
$peNDF_{1.18}$, % of DM	26.0	26.4	24.1	26.7	0.40	0.92	<0.01

[1]Percentage inclusion of ground licuri. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects. [3]Standard error of the mean. [4]Particle size distribution of mixed diets or orts was measured using the Penn State Particle Separator (Kononoff et al., 2003); $pef_{8.0}$ and $pef_{1.18}$ = physical effectiveness factor determined as the proportion of particles retained on 2 sieves (Lammers et al., 1996) and on 3 sieves (Kononoff et al., 2003), respectively; $peNDF_{8.0}$ and $peNDF_{1.18}$ = physically effective NDF determined as NDF content of diets multiplied by $pef_{8.0}$ and $pef_{1.18}$, respectively.

However, The proportion of particles retained on the 8.0-mm sieves, pan, **pef$_{1.18}$** value and, peNDF$_{8.0}$ had no effect. In the diets consumed (adjusted for particle size of orts), the proportion of particles retained on the 19-mm, 8-mm sieves, value **pef$_{8.0}$**, value **pef$_{1.18}$** and, peNDF$_{8.0}$ showed a increasing linear effect (P<0.05) with the inclusion of ground licuri (Table 4). The proportion of particles retained on the pan showed a decreasing linear effect (*P*>0.05). The particles retained on the 1.18-mm sieves and peNDF$_{1.18}$ showed no effect.

Table 5. Least square means of the chewing activity of lambs fed increasing levels of ground licuri

Item	Tratements[1]				SEM[3]	*P*[2]	
	0	5	10	15		L	Q
Eating							
Min.day^{-1}	250	186	198	255	22.51	0.69	0.02
Min.g^{-1} of DM	0.26	0.22	0.23	0.39	0.20	0.02	0.01
Min.g^{-1} of NDF	0.68	0.60	0.71	1.01	0.10	0.02	0.06
Min.g^{-1} of peNDF$_{8.0}$[4]	2.84	2.49	2.64	3.49	0.36	0.21	0.12
Min.g^{-1} of peNDF$_{1.18}$[4]	1.01	0.87	1.00	1.37	0.14	0.05	0.07
Ruminating							
Min.day^{-1}	374	412	394	419	23.40	0.14	0.58
Min.g^{-1} of DM	0.41	0.49	0.46	0.63	0.03	<0.01	0.21
Min.g^{-1} of NDF	1.10	1.32	1.40	1.62	0.08	<0.01	0.98
Min.g^{-1} of peNDF$_{8.0}$[4]	4.49	5.44	4.99	5.89	0.37	0.03	0.94
Min.g^{-1} of peNDF$_{1.18}$[4]	1.59	1.89	1.90	2.31	0.14	<0.01	0.71
Chewing							
Min.day^{-1}	623	597	593	673	32.89	0.33	0.11
Min.g^{-1} of DM	0.66	0.72	0.69	1.02	0.06	<0.01	0.02
Min.g^{-1} of NDF	1.78	1.92	2.11	2.76	0.14	<0.01	0.07
Min.g^{-1} of peNDF$_{8.0}$[4]	7.43	8.00	7.82	9.02	0.55	0.07	0.58
Min.g^{-1} of peNDF$_{1.18}$[4]	2.63	2.78	2.97	3.55	0.21	<0.01	0.33
Idle (min.day^{-1})	816	843	846	767	33.84	0.33	0.11

[1]Percentage inclusion of ground licuri. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects. [3]Standard error of the mean. [4]peNDF$_{8.0}$ and peNDF$_{1.18}$ = physically effective NDF determined as NDF content of mixed diet multiplied by pef$_{8.0}$ and pef$_{1.18}$, respectively. pef$_{8.0}$ and pef$_{1.18}$ = physical effectiveness factor determined as the proportion of particles retained on 2 sieves (Lammers et al., 1996) and on 3 sieves (Kononoff et al., 2003), respectively.

Eating activity (min.day^{-1}) showed quadratic effect (*P*=0.02) as dietary ground licuri content increased. However, ruminating and total chewing activity time (eating +

ruminating) no showed effect. Eating, ruminating and total chewing time per gram of DM and NDF intake increased linearly (P<0.05) with ground licuri inclusion in the diet. In the same way, ruminating and total chewing per gram of $peNDF_{8.0}$ and $peNDF_{1.18}$ intake had same effect (P<0.05). However, idle and eating $peNDF_{8.0}$ intake had no effect, but eating $peNDF_{1.18}$ intake increased linearly (P=0.05).

3.3 Nitrogen balance and microbial protein synthesis

Nitrogen excretion via feces showed a decreasing linear effect (P<0.05). In the same way, microbial protein synthesis (MPS) and efficiency microbial protein synthesis (EMPS) showed a decreasing linear effect (P<0.05). However, showed no effect in the N intake, feces N, absorbed N, and retained nitrogen (Table 6).

Table 6. Least square means of nitrogen balance and microbial protein synthesis of lambs fed increasing levels of ground licuri

Item	Treatments[1]				SEM[3]	P[2]	
	0	5	10	15		L	Q
Nitrogen balance (g day^{-1})							
Intake N	24.28	24.12	26.24	19.24	1.81	0.13	0.08
Fecal N	6.36	5.92	6.32	4.46	0.48	0.02	0.15
Urinary N	9.38	6.38	12.46	7.10	1.50	0.09	0.48
N absorbed	17.94	18.24	19.92	14.80	1.52	0.27	0.09
N retained	8.52	11.88	7.46	7.72	2.17	0.49	0.48
g kg $BW^{0.75}$ day^{-1}	0.70	1.00	0.62	0.66	0.17	0.51	0.44
MPS[4] g day^{-1}	74.46	67.27	63.16	33.21	8.21	<0.01	0.17
EMPS[5] g of N/g of TDN	116.17	116.18	105.88	70.95	13.12	0.02	0.19

[1]Percentage inclusion of ground licuri. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects. [3]Standard error of the mean. Linear effect (L). Quadratic effect (Q). [4]Microbial protein synthesis. [5]Efficiency MPS.

3.4 Blood parameters

Inclusion of licuri in the diets did not showed effect in the blood serum concentration of creatinine, albumin, triglyceride, NEFA, alanine aminotransferase (ALT), and aspartate aminotransferase (AST); however, serum urea concentrations had increasing linear effect; in addition serum glucose showed opposite response, between treatments P<0.05 (Table 7).

Table 7. Least square means of blood parameters of lambs fed increasing levels of ground licuri

Item	Treatments[1]				SEM[3]	*P*[2]				
						Treat		Time		Treat x Time
	0	5	10	15		L	Q	L	Q	
Creatinine (mg/dL)	1.06	1.05	1.04	1.08	0.08	0.89	0.75	<0.01	0.06	0.77
Albumin (g/dL)	3.84	3.84	3.73	3.74	0.09	0.33	0.97	0.11	0.56	0.70
Urea (mg/dL)	42.03	43.76	46.86	48.29	1.50	0.01	0.29	<0.01	<0.01	<0.01
Glucose (mg/dL)	99.85	94.27	97.83	93.59	1.50	0.03	0.66	<0.01	0.01	0.83
NEFA[4] mmol/L	0.94	0.94	0.94	0.87	0.04	0.27	0.43	0.05	0.05	0.83
Triglyceride (mg/dL)	108.17	106.45	110.35	100.85	2.83	0.17	0.18	<0.01	<0.01	0.41
ALT[5] (UI/L)	20.50	14.35	18.80	18.60	2.50	0.91	0.25	0.92	0.40	0.58
AST[6] (UI/L)	100.25	97.15	101.80	69.65	9.84	0.55	0.65	0.07	0.44	0.74

[1]Percentage inclusion of ground licuri seed. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects of the treatments (Treat), time and the treatment by time interaction (Treat x Time). [3]Standard error of the mean. [4] Non-esterified fatty acids. [5]Alanine aminotransferase. [6]Aspartate aminotransferase.

The blood serum concentration of urea, glucose, NEFA and triglyceride showed a quadratic effect; however, serum creatinine concentrations had a linear effect in sampling times ($P<0.05$). The treatment by time interactions had no effect for all blood metabolites evaluated in this study, except for concentration of urea in blood serum ($P<0.01$).

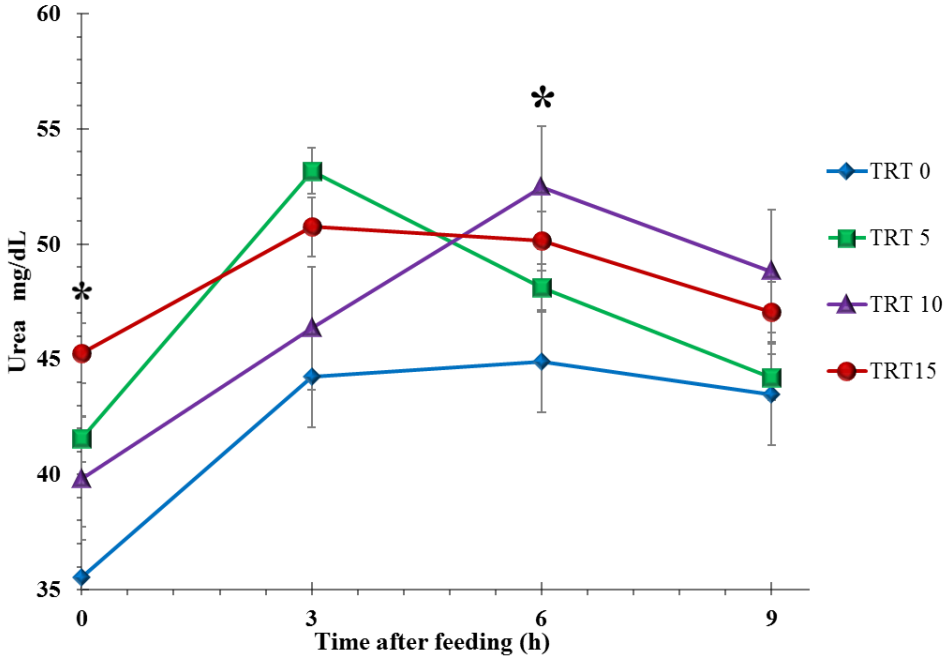

Figure 1. Temporal change of serum urea concentration of feedlot lambs fed ground licuri. TRT; Percentage inclusion of ground licuri

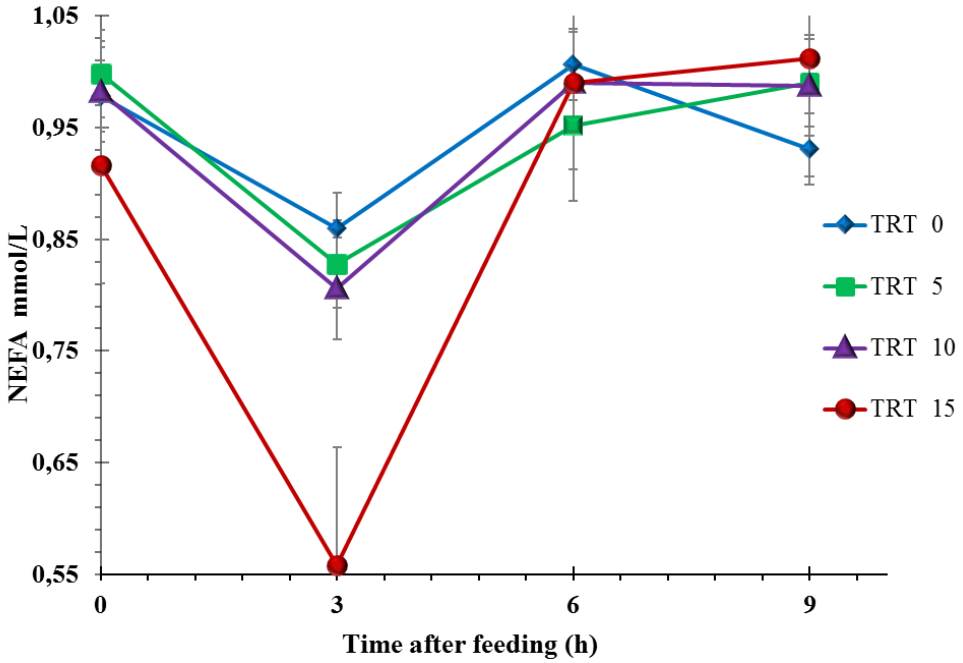

Figure 2. Temporal change of serum NEFA concentration of feedlot lambs fed ground licuri. TRT; Percentage inclusion of ground licuri

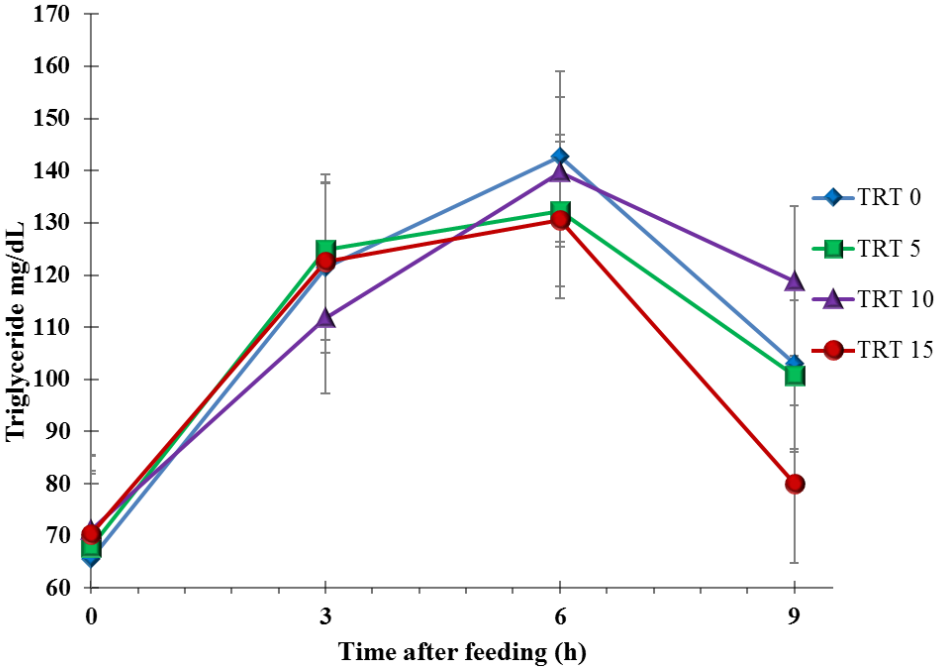

Figure 3. Temporal change of serum triglyceride concentration of feedlot lambs fed ground licuri. TRT; Percentage inclusion of ground licuri

3.5 Performance and characteristics of the Longissimus dorsi muscle

The average initial weight of the lambs was 20.89 kg (± 3.97 BW kg). The licuri included in the diets had a decreasing linear effect in all the performance variables and in the fat thickness ($P<0.05$). However, the inclusion of licuri had quadratic effect in the feed conversion (Table 8).

The inclusion of ground licuri in the diets had no effect ($P>0.05$) on the physical, chemical, and color characteristics of the *Longissimus dorsi* muscle (Table. 9)

Table 8. Least square means of performance parameters of lambs fed increasing levels of ground licuri

Item	Treatments[1]				SEM[3]	*P*[2]	
	0	5	10	15		L	Q
Initial Weight (kg)	20.61	21.14	21.62	20.21	---	---	---
BW[4] at 25 day (kg)	27.36	27.12	26.66	25.19	0.61	0.01	0.28
BW[4] at 75 day (kg)	36.68	33.46	34.76	32.26	0.77	<0.01	0.62
WG[5] (kg)							
0 to 25 day	6.52	6.23	5.77	4.22	0.61	0.01	0.31
0 to 75 day	15.54	12.28	13.56	11.14	0.74	<0.01	0.57
ADG[6] (g.day^{-1})							
0 to 25 day	250	239	222	162	23.41	0.01	0.30
0 to 75 day	215	170	188	154	10.42	<0.01	0.58
Fat thickness (mm)	1.19	1.41	1.17	0.81	0.12	0.02	0.03
FCR[7]	4.44	5.04	4.68	4.61	0.16	0.83	0.05

[1]Percentage inclusion of ground licuri seed [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects [3]Standard error of the mean [4]Body weight [5]Weigth gain [6]Averge daily gain [7]Feed conversion ratio.

Table 9. Least square means of the chemical and physical characteristics of the Longissimus dorsi muscle of lambs fed increasing levels of ground licuri

Item	Treatraments[1]				SEM[3]	*P*[2]	
	0	5	10	15		L	Q
Chemical composition (%)							
Moisture	71.0	71.7	71.1	72.3	0.55	0.19	0.64
Ash	1.21	1.17	1.14	1.11	0.03	0.07	0.87
Crude protein	26.2	24.9	25.2	24.9	0.43	0.07	0.30
Total lipids	1.76	2.03	2.09	1.77	0.29	0.95	0.34
Color at 30 minutes (CIE)[4]							
L* - Luminosity	35.6	36.8	36.3	37.0	0.71	0.25	0.78
a* - Red	20.4	20.8	20.4	20.0	0.37	0.41	0.30
b* - Yellow	7.24	7.89	7.17	7.09	0.49	0.59	0.46
Chroma	21.7	22.3	21.7	21.3	0.43	0.39	0.25
Hue angle	19.5	20.7	19.1	19.5	1.17	0.75	0.72
Physical characteristics							
WHC[5] (%)	34.4	35.5	30.9	34.9	1.96	0.78	0.49
WLC[6] (%)	13.8	11.7	13.3	9.5	2.36	0.28	0.71
Loin shear force (kg.cm^{-2})	1.30	1.30	1.29	1.16	0.11	0.78	0.88

[1]Percentage inclusion of ground licury seed. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects. [3]Standard error of the mean. [4]CIE: Commission Internationale de l'Eclairage. [5]Water-holding capacity. [6]Water cooking loss.

4.0 DISCUSSION

4.1 Intake and digestibility

The inclusion of licuri in the experimental diets increased non-forage NDF, EE, and decreased the NFC, which resulted in a linear decreasing on the intake of DM and, consequently, also a linear decreased on the OM, CP, NDF, NFC and TDN intake, except on the EE intake that linear increased (P>0.05). This effect on nutrients intake suggest mainly the effect of filling caused by the increase of the in 28.4 % of NDF in the diet with 15% of ground licuri. The factors that affect fill, including particle size, chewing frequency and effectiveness, particle fragility, indigestible NDF fraction, rate of fermentation of the potentially digestible NDF, and characteristics of reticular contractions (ALLEN, 1996). In contrast, others non-forage fiber source (NFFS), as corn gluten feed and brewers' grains rice bran, beep pulp, corn distillers dried grains, soybean hulls, and corn gluten, possess fiber that ferments and passes rapidly from the rumen (COPPOCK, 1987) and, possibly require to increase dietary NDF concentrations due less fiber is retained in the rumen to stimulate chewing and saliva secretion (BELYEA et al., 1989). Similarly, Clark and Armentano, (1997) described wich as the mean particle length or forage NDF content increases, ruminal retention time and the forage mat effect increases, which may slow the passage rate of NFFS, thus enhancing fiber digestibility

The lipid profile of licuri predominantly shows medium chain fatty acids (MCFA) mainly lauric and myristic acids (BAUER et al., 2013), and the palatability of MCFA decreases DMI (HRISTOV et al., 2011). The MCFA are absorbed in the abomasum, then going to the portal hepatic system, these FA have a high propensity for oxidation, to behave in a similar way as glucose (MARTEN et al., 2006). However, although the inclusion of licuri increases the EE by 41.56% between control diet and 15% of inclusion, this does not exceed 5% that could affect the intake (PALMQUIST and JENKINS, 1980).

Another factor that could have negatively influenced intake is the physiological maturity of lambs reached during the experiment.

The ruminal digestibility of a nutrient is a function of the potentially digestible fraction and is affected by rates of digestion and passage (FIRKINS, 1997). The increase

in EE intake increases its digestibility as a function of the dilution of the endogenous EE and by the intestinal digestibility of the FA that is inversely related to the melting point of the FA (NRC, 2001).

The decrease on the digestibility coefficient of NFC resulted of the decreases of same intake, which increased the participation of metabolic fractions in the fecal excretion, increasing in the apparent indigestible fraction intake of NFC (VAN SOEST, 1994). Comparing the diets 0% and 15% of licuri, even the EE intake and digestibility increased with the increase of licuri, it corresponded to account in TDN 27.0 g of energy, when the NFC energy decreased correspond to 105.0 g TDN, approximately decreased four times energy in the licuri diet. Likewise, the decreases in the NFC affect more than EE increased when ground corn is replaced by licuri in the diets to lambs.

4.2 Physical effectiveness NDF and chewing activity

The particles retained on the 1.18-mm sieve of the orts decreased; in contrast, in the consumed diets (estimated) increased (Table 4). The increase of particles retained on the 1.18 mm sieve of orts is due mainly to the endocarp of the ground licuri, which increases as the licuri is included in the diets, a hard, dense and undigestible structure; but in the consumed diet (1.18-mm) it was similar; moreover, the physical effectiveness factors (pef; 8.0- and 1.18), and $peNDF_{8.0}$ of the consumed diet (adjusted) increased, this finding suggest that long particles were preferred by lambs and may be associated with the diet selection. However, in this study the NFC intake decreased, suggest that the pH was not affected negatively. Sheep counteract the effects of direct change of the ruminal environment by adjusting the quantities of feeds selected as a choice to maintain ruminations, and the ruminal conditions (COOPER et al., 1996). The animals can select the diet with a particular sensorial profile due to its metabolic effects and can adjust their diet quantitatively and qualitatively to meet their nutrient requirements (FORBES, 2007). The increasing dietary peNDF resulted in decreased DMI and, consequently, the decreased of nutrient intakes (OM, CP, NFC, TDN; Table 3). The voluntary DMI and ruminal filling is related to the bulk density of forages (WATTIAUX, 1990), which reflected negatively on the intake, and performance in this study.

Results found by Silva et al. (2014) showed the low in vitro NDF digestibility 26.77% and high lignin (22.69 %) content to licuri fruit. In comparison, to Tifton hay in

vitro digestibility DM is 58.7%, NDF disappearance rate at 48 hours is 55.7% and, lignin content 4.8% (MANDEBVU et al., 1999). Those results show even the peNDF of Tifton hay and licuri were similar, the NDF digestibility in licuri are lower and would explain the decreased of intake, by ruminal physical limitation. The effectiveness of the NDF can increase ruminal retention time and allowed complete digestion of the potentially fermentable fraction, increasing digestibility (GRANT, 1997), but in this currently study, it was not observed.

The peNDF is related to DMI, particle size, particle shape, fragility, moisture, type of preservation, and ratio of eating time to ruminating time (MERTENS, 1997). The physical and chemical nature of dietary NDF largely drives chewing activity (GRANT, 1997). The total chewing time is strongly influenced by the forage content and size of particles in the food (VAN SOEST, 1994). Total chewing and ruminating time were linearly increased with increasing dietary peNDF (YANG and BEAUCHEMIN, 2006). Beauchemin (1991) found that the proportion of NDF in the diet increased, each unit of DM was chewed for a longer period of time mainly during eating, which resulted in loss ruminating time per unit of NDF consumed. Therefore, it is well recognized that increasing peNDF of diets increases ruminating activity (LU, 1987; YANSARI A. T. et al., 2004; ZHAO et al., 2010). We expected that, with the increase of NDF in the diets, eating and chewing would also increas; however, in this study the licuri inclusion increased peNDF (8.0, 1.18), DMI decreased and we observed that eating time (min.day^{-1}) showed response quadratic, but ruminating time (min.day^{-1}) was not change, the lack of effect on ruminating and total chewing times can be explain by lower intake with licuri inclusion. Yang and Beauchemin (2005) reported, that the increase forage particle length increased intake of peNDF but did not affect DMI and NDF. Contrary, Zhao et al. (2010) in a study with goats, increasing forage particle length increased intake of peNDF, but decreased DMI linearly ($P = 0.05$).

Ruminating time oscillate between 374 to 419 min.day^{-1}. Deswysen et al. (1978) reported that texel lambs spent between 494 and 571 min.day^{-1} ruminating. Similar values (555 and 579 min.day^{-1}) were reported by Baumont et al. (1990) for same breed. However, licuri inclusion resulted in increased of eating, ruminating, and total chewing times per gram of DM and NDF intake, as well as ruminating and chewing time per gram of peNDF (8.0, 1.18), these increases was no altered the total time spent ruminating and

chewing (min.day^{-1}). Rumination time and chews per unit of consumed fiber can be considered to be indices of the fragility or susceptibility of the cell wall to breakdown (BEAUCHEMIN and BUCHANAN-SMITH, 1991). Ruminants may possess an adaptive mechanism whereby they ruminate more effectively (more chews/kg of forage NDF intake) under conditions of limited amounts of effective forage NDF (GRANT, 1997). The majority non-forage NDF sources decreased the rumination time (min/day), which require adjust in the forage fiber for maintenance of chewing activity (CLARK and ARMENTANO, 1993, 1997; CLARK and ARMENTANO, 1993; COPPOCK, 1987), with possible exceptions of whole cottonseed, cottonseed hulls, and citrus pulp that possess greater physical effectiveness (BELYEA et al., 1989; MOONEY and ALLEN, 1997; CLARK and ARMENTANO, 1993). In addition, Beauchemin and Buchanan-smith (1991), studying eating behavior with cows, reported that rumination time increased substantially between 26 and 30% NDF, although this parameter did not increase between 30 and 34 % NDF. However, Beauchemin (1991) and Beauchemin et al (1991) observed that rumination time was not different for diets containing 29 to 32% NDF.

4.3 Nitrogen Balance and microbial protein synthesis (MPS)

The positive N balance of all treatments indicates balance between the dietary protein and energy. As a proportion of N intake, N retained ranged from 28.4 to 49.2% and was not affected by treatments despite no changes in N intake and decreased fecal N excretion (42%) at treatment with licuri inclusion; therefore, is possible which increasing levels of licuri in the diets are related with compensatory effect or better N utilization.

The MPS in the rumen provides up to 80% absorbable protein in the small intestine (STORM and ØRSKOV, 1983). The total amount of microbial protein flowing to the small intestine depends on supply of adequate amounts and type of CHO as an energy source for the synthesis of peptide bonds (BACH et al., 2005; CALSAMIGLIA et al., 2010). The 15% licuri inclusion increased EE intake in 88.0% and decreased the intake of NFC and TDN by 33.5% and 23.5%, respectively, compared to the control diet, which negatively affected the efficiency and production of microbial protein. Dietary fat is energy dense but does not provide fermentable energy for microbial growth in the rumen (STERN et al., 1994), and the substitution of fermentable carbohydrates by EE can reduce the synthesis of microbial protein and consequently animal protein requirements

may not be met (GOULAS et al., 2001). Ruminal synchrony between ruminaly digestible protein and energy sources is useful to simplify the description of energy and protein supply to rumen microbes (JOHNSON, 1976). The increase of NDF and lignin in the diets with inclusion of ground licuri, renders the degradation of energy and N-yielding substrates required for microbial growth are not available or synchronized (REYNOLDS and KRISTENSEN, 2008). The degree of synchrony will only influence microbial protein synthesis with diets containing high levels of readily fermentable carbohydrate (DEWHURST et al., 2000). The optimum ratio of NFC to ammonia N has not yet been determined (BACH et al., 2005). In contrast to our results, Yang et al. (2003), Rode et al., (1985), Yang and Beauchemin, (2005) reported that the EMPS improved with increasing peNDF in the diet due to an increased amount of organic matter fermented in the rumen. The *in vitro* NDF digestibility of licuri is low 26% (Silva et al., 2014), decreasing the availability of organic matter fermentable. Efficient utilization of degraded dietary nitrogen requires that the energy from the fermentation of dietary OM must be supplied at a rate which matches the synthetic abilities of the rumen microbes (STERN and HOOVER, 1979).

4.3 Performance and carcass characteristics

The inclusion of licuri in the diets showed decreasing linear effect ($P<0.05$) on all variables evaluated (Table 8) on the animal performance, these responses are support by the decrease of 25.1% on the DMI between the lowest (control) and the highest level on licuri inclusion. The decrease in DMI led to a lower intake of CP, TDN and other nutrients, as well as a decrease in the supply of microbial proteins, negatively affecting performance and fat thickness (FT) of the lambs. The average daily gain (ADG) decreased linearly; however, the highest ADG was achieved in the period of 0 to 25 days (oscillate 162 to 250 $g.day^{1}$) compared to period of 0 to 75 days (oscillate 154 to 215 $g.day^{1}$), this response was expected due to the negative relationship between ADG and animal maturity. Despite the fact that ADG obtained of the experimental diets in this study was 171 $g.day^{1}$, a value slightly higher to 160 $g.day^{1}$ reported by Costa et al. (2016) for Santa Ines x Dorper lambs fed licuri cake; however, the FT was lower in our treatments, which decreased linearly ($P<0.05$), and ranges between 1.41 to 0.81mm. On

the other hand, Santos et al. (2015) reported a quadratic effect ($P<0.05$) in the performance without changes in the FT of *longissimus* muscle when lambs were fed licuri cake. Urano et al. (2006) and Ferreira et al. (2011) reported FT values of 1.5 and 1.9 mm, respectively; and Costa et al. (2016) reported 3.4 mm for crossbred Santa Inês x Dorper lambs with similar slaughter weights. Our responses may be due to the lowest intake of CP and TDN as the ground licuri is included in the diet, that resulted in decreased muscle deposition and lower accumulation of subcutaneous fat. The main substrate for lipogenesis is acetate from rumen fermentation of carbohydrates (VERNON et al, 2001), the deposition of adipose tissue result from the maintenance of a fine balance between energy intake and energy expenditure, energy storage and energy mobilization (NÜRNBERG et al., 1998). The FT is influenced by sex (female greater than males), breeds and crossbreeds (FERNÁNDEZ et al., 1997). Fat gives nutritional value, tenderness, flavor, succulence and protection to the meat during chilling; however, when in excess, it has a negative impact (WOOD et al., 2004). The FT measurements of *longissimus* muscle is predictors of carcass lean content, subcutaneous fat and subcutaneous plus intermuscular fat (WOOD and MACFIE, 1980). Fat depots amounts and composition influence lamb carcass and meat quality (MELTON, 1990, JUÁREZ et al., 2008). Intramuscular, intermuscular and subcutaneous fats significantly affect flavour, juiciness and texture of meat (WOOD, 1984).

In the current study, control lambs had the highest weight gain and best feed conversion ratio (FCR); however, average FCR obtained was 4.69, better value than those reported by Costa et al. (2016) and SCR Felix et al. (2016) in crossbred Santa Inês x Dorper lambs and Francisco et al. (2015) in White Merino lambs that were around than 6. The best FCR is attributed mainly to the higher efficiency of utilization of the absorbed nutrients.

The average content of moisture, crude protein, fat and ash, were 71.7, 25.0, 1.9 and 1.15 %, respectively. Fat and meat colour have great influence on the purchase decision of consumers (PAGE et al., 2001; RIPOLL et al., 2008). The intramuscular moisture content increases as intramuscular lipids decrease (OKEUDO and MOSS, 2005). High correlation coefficients were observed between intramuscular lipid content and moisture content (-0.78) (OKEUDO and MOSS, 2005). The moisture content is related to the juiciness, which is the ability of the muscle to release its water (initial

juiciness) and the infiltrated fat content (sustained juiciness) (DRYDEN and MARCHELO, 1970). The intramuscular fat deposits in and around the muscle fibers and is an important indicator of juiciness, texture and flavor of the meat (SAÑUDO et al., 2000), and fat content affects the palatability of meat (HOFFMAN et al., 2003). The meat fat can trap moisture in muscle, improving juiciness (WOOD et al., 2004). The intramuscular fat content affects the palatability of meat (HOFFMAN et al., 2003). The licuri inclusion in the current study decreased concentration of NFC in favor of fibrolytic bacteria activity which lead to a greater formation of acetate, the acetate lead to higher fat deposition in the carcass (ALLEN et al., 2009), however, this effect was not observed. The meat fat was lower than that found by Sen et al. (2004) but similar to that reported by Pérez et al. (2002). The meat protein was highest that observed in several studies (GONZÁLEZ et al., 2014; D'ALESSANDRO et al., 2013). Muscles with the highest protein content was characterised by a lower fat content (HOFFMAN et al., 2003; KEMP et al., 1976), agree with our study.

The meat from lambs fed with different levels of inclusion of ground licuri showed similar lightness (L* value). Greater L* values denote lighter/paler meat and greater a* and b* values indicate a more red and yellow color, respectively (MILTENBURG et al., 1992). Our treatments displayed average L* value of 36.4, this values, according to, Hajji et al. (2016) is a light-coloured and acceptable meat. The meat with a lightness value equal to or above 34 was acceptable on average and above 44 was acceptable by 95% of consumers (KHLIJI et al., 2010). Animals with greater fat thickness had a tendency to produce lighter-colored meat (KADIM et al., 1993). The similar percentage of intramuscular fat deposition in meat colud be a reason for similar color, espciallly in the L* index, which was lower to the findings of Asadollahi et al. (2017). The meat becomes darker as the animals age (SANTOS-SILVA et al., 2004); likewise, the myoglobin concentration of muscles increases with age (LEDWARD and SHORTHOSE, 1971). The reactions of the heme pigments of meat, myoglobin and hemoglobin, are important in determining the colors (FOX, 1966). The oxymyoglobin heme iron undergoes a univalent oxidation to the ferric oxidation state, fresh red muscle loses its attractive, bright, cherry-red surface appearance and becomes brown (GIDDINGS, 1977). On the other hand, the L* value was positively and linearly correlated with the proportion of licuri cake in the diet of young bulls (DE GOUVÊA et al., 2016). Slaughter live weight is more important

factor than breed that influence meat color, and as slaughter weight increases, meat lightness decreases (SAÑUDO et al., 2007). The heavier carcasses are darker (L*) and less tough, and do not present any difference in Water-holding capacity (WHC) than the meat of medium weight carcasses (SAÑUDO et al., 1996). The intensity of the red color, termed saturation or chroma, declines gradually as the display period progresses (WOOD et al., 2008). A change in muscle color suggested a link between lipid oxidation, vitamin E concentration and color (WOOD et al., 2008). The high a∗ value is associated with raised pigmentation depending on increased muscle activity and live weight of the lambs (KARACA et al., 2016). Other factor ,the high pH value, have adverse effects on colour (darker), tenderness, flavour and storage life (HOPKINS et al., 2007; PRIOLO et al., 2002; SAÑUDO et al., 2007). On the other hand, Fogarty et al. (2000) didn't reported differences in the loin colour of meat from of diverse range of genotypes.

Water holding capacity (WHC) is the ability of fresh meat to retain its own water (PEARCE et al., 2011). The muscular myofibril matrix is the main structural unit responsible for the water content in the meat (HAMM, 1986). Therefore, changes in WHC are very sensitive indicators of changes in the structure of myofibrillar proteins (HAMM, 1961; HONIKEL et al., 1986). The WHC is correlated with mainly sarcoplasmic protein solubility and myofibrillar protein solubility (JOO et al., 1999). Likewise, it has been related to important meat organoleptic properties such as juiciness and tenderness (SAÑUDO et al., 1996). Beriain et al. (1997), reported WHC value of 24.63 and 23.01 % for lacha and rasa aragonesa (36 kg BW), respectively. Likewise, Velasco et al. (2004), reported 17.9 % of WHC for Talaverana-breed lambs. However, in the current study the WHC oscillate between 30.9 to 35.5%, this response suggests which higher WHC.

In this study, the average water cooking loss (WCL) was 12.1 % with 1.91 % average meat fat. Hoffman et al. (2003), reported 23.04 and 17.76 % of water cooking loss for Dormer x Merino and Suffolk x Merino lambs breeds, respectively, both with higher intramuscular fat which of the present study. Higher WCL were reported when carcass had increased fat content (SCHONFELDT et al., 1993). The presence of intramuscular fat loosens up the microstructure of the meat, causing more water to be entrained (FAUCITANO et al., 2011). However, Hajji et al. (2016) reported that higher meat fat deposition limits the WLC in fattier lambs from the three breedsss, the mean value of the WCL (14.49 %) was slightly higher than the value of the present study. The

WLC is negatively correlated with juiciness and is a significant predictors of for overall liking and flavour (HOPKINS et al., 2006) and, positively correlated with moisture content ($r = 0.55$, $P < 0.001$) (OKEUDO and MOSS, 2005). In addition, the WHC of all methods depends on the pH of the meat, which changes after death due to the formation of lactic acid (HONIKEL, 2004).

The loin shear force average was 1.26 kg, this values were lower than 3.41, 3.01 and 6.76 kg reported by Bessa et al. (2005), Santos-Silva et al. (2004) and Hoffman et al. (2003), respectively. Shear force values below 11 kg force are regarded as indicating an acceptable tenderness and values less than 8 kg force are highly acceptable (DEVINE et al., 1993). However, Shackelford et al. (1991), reported values greater than 5.5 kg of shear force are often considered as hard by consumers. The meat quality (overall liking) declined as shear force and age increased, and as intramuscular fat percentage (HOPKINS et al., 2006). Shear force was positively associated with cooking loss ($r = 0.42$, $P<0.001$), but negatively with intramuscular fat content ($r = -0.55$, $P < 0.001$) (OKEUDO and MOSS, 2005).

In general, all levels of ground licuri inclusion in the diet produced meat of good quality, despite the decreasing effect on animal performance.

4.4 Blood parameters

The inclusion of licuri had no effect on serum creatinine and albumin. Blood creatinine is a product of nitrogen metabolism and is considered an index of endogenous protein catabolism and also used to asses renal function (BIANCHI et al., 2014). The albumin is synthetized in the liver and its synthesis depending on nutritional status (WOLF, 1999). The albumin can reflect protein deficiency problems over a period of a month or two (VAN SAUN, 2000).

Serum urea showed increasing linear effect among treatments, although the diets were isonitrogenous. Higher amounts of dietary rumen carbohydrate are associated with increased sequestration of NH_3-N into microbial protein (HRISTOV et al., 2005). The lower NFC availability, should reflect in the least sequestration of urea increasing that in the blood and decreasing MPS in the 15% of licuri diet.

Serum urea ($mg.dL^{-1}$) has a difference in the 0 and 6 hours after morning feeding. The concentration of urea is lowest in the 0% of licuri in the diet (Figure 1). Except ALT

and AST, all the blood metabolites assessed showed quadratic effect ($P<0.05$) at different collected time after feeding, postprandial responses expected by the nutrients absorption.

The glucose showed decreasing linear effect associated to the decreasing of NFC with licuri inclusion. Propionate is rapidly produced by rumen microbes, primarily by fermentation of starch; however, starch escaping fermentation in the rumen can be digested and absorbed in the small intestine providing glucose and lactic acid as absorbed fuels (ALLEN and BRADFORD, 2012). It is assumed that propionate availability limits glucose production, increasing glycogenic demands on aminoacids (DRACKLEY, J. K. et al., 2001). However, glucose is synthetized by hepatic and renal tissues due net glucose absorption from the hepatic portal-drained viscera is negligible or negative (REYNOLDS et al., 1988).

In addition, in ruminants, others substrate can be used for synthesis of glucose and glycogenic aminoacids are only partially oxidized in the liver and they reasoned that the pathways to the end-products, glucose and urea, could be viewed as ATP utilizing–ADP regenerating systems which allow the continued catabolism of dietary amino acids (WATFORD, 1999). This finding supports the increase of urea previously described.

The serum TG and NEFA showed quadratic effect at different times after feeding ($P>0.05$). NEFA is a indicative of body lipid storage oxidation, and that oxidation of NEFA might suppress feed intake and satiety (PALMQUIST and CONRAD, 1978). However, throughout the experimental period, licuri inclusion has not effect on serum TG and NEFA. The blood parameters in this study were kept within normal references values, except for the serum concentration of TG that was higher in all treatments, according to Kaneko et al. (2008).

Though the increases in serum AST are considered less specific for liver disease than increases in serum ALT, both are observed with hepatocellular inflammation and injury (KANEKO et. al., 2008). The results showed that licuri ground in the diets has no toxic effect on lamb livers (Table 7).

5.0 CONCLUSION

Ground licuri seed included replacing corn ground as an energetic feed, is not recommended. The inclusion of ground licuri increased the concentrations of NDF and decreased NFC in the diets, causing a negative impact on nutrients intake, microbial protein synthesis and performance concomitantly.

The chemical and physical meat quality traits were not affected by treatments. The peNDF is similar to Tifton hay and shows the potential use of ground licuri as NDF non forage and subject to further studies in which they should make adjustments mainly of NDF and EE to determine the optimum levels to be included in the diets of lambs.

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CHAPTER II

Effect of ground licuri on the ruminal metabolism of lambs

Effect of ground licuri on the ruminal metabolism of lambs

ABSTRACT

The objective this study was to evaluate the effects of the supplementation of licuri ground 0; 4; 8; 12 and 16% DM basis of the diets on intake, digestibility, nitrogen balance, MPS, EMPS, ruminal parameters, and blood parameters. Five crossbred Dorper x Santa Ines, non-castrated males ram, with average body weight of 42.5 kg were used in a 5 × 5 Latin square design, housed in rams feedlot cage, with five periods of 16 days, that included 6 days samples collection. The rams were fed twice daily, at 8:00 h and 16:00 h, ensuring a 10% surplus and water supply was *ad libitum*. The lambs were fed a mixture of Tifton hay (26 %), sisal silage (28 %) and a concentrated mixture (47 %). The diet had a 53:47 forage to concentrate ratio. Significance was declared at $P \leq 0.05$. The ground licuri inclusion did not affect ($P>0.05$) nutrient intake, nutrient digestibility, rumen fermentation parameters, nitrogen balance, MPS, EMPS, and blood parameters. The pH and concentration of NH_3-N, acetate, propionate, butyrate, and total VFA, showed quadratic effect ($P<0.05$) among collection times (0, 2, 4, 6 and 8 hours) after feeding. The ground licuri inclusion showed no effect ($P>0.05$) on the treatments x time interactions of the rumen fermentation parameters. The ground licuri inclusion increased the NDF, EE, and decreased NFC as was included in the diets, and thus, contrary to we expected, the parameters evaluated in this study were not affected. The ground licuri can substitute to ground corn up 16 %.

Abbreviations*:* DM, dry matter; DMI, dry matter intake; CP, crude protein; NDF, neutral detergent fiber; peNDF, physically effectiveness NDF; NFC, non-fibrous carbohydrates; ADG average daily gain; NEFA *Non-esterified fatty acids*; TG Triglyceride; MPS, microbial protein synthesis; EMPS efficiency microbial protein synthesis

Key words: Ram, digestibility; performance, rumen fermentation parameters; blood parameters.

1.0 INTRODUCTION

Alternative feeds are mainly used as a source of low-cost nutrients or for their high availability to the sheep farmers. The success of their use depends on the nutritional characteristics, palatability and how they affect intake and ruminal fermentation. The licuri (*Syagrus coronata*) is a native palm of the region Northest of Brazil, also found at sheep farms in the Bahia semiarid region. Its fruit is used in human feeding and as agroindustrial residues (by products) that have ben used to feed ruminants.

The licuri seed is characterized by its high content of medium chain fatty acids MCFA (60%), mainly lauric (43%) and myristic (14%) fatty acids present in refined and the pressed oil (BAUER et al., 2013), furthermore, the licuri cake has 48.3% of NDF (COSTA et al., 2016).

Fat is often added to diets to increase energy density; however, fat must be carefully considered because MCFA can negatively affect energy intake (ALLEN, 2011). The physical-chemical characteristics and the concentration of the NDF is closely related to intake potential (BUXTON, 1996), and variation in dry matter digestibility is related primarily to the concentration and digestibility of NDF (MERTENS, 1969).

The inclusion of licuri cake in the diets of feedlot lambs increases the concentration of ether extract (EE), NDF, and did not influence the dry matter intake and the average daily weight showed quadratic effect $P<0.05$ (SANTOS et al., 2015), however, Costa et al. (2016) reported that intake and the total weight gain decreased linearly with the inclusion of licuri cake in the diets of lambs. Therefore, by the nutritional characteristics of licuri and the absence of studies using ground licuri seed in the diet of lambs, the aim of this study is to evaluate the effects of inclusion of licuri seed on the diet of lambs, intake, digestibility, and ruminal microbiology in feedlot lambs.

2.0 MATERIAL E METHODS

The study was carried out at the Experimental Farm of São Gonçalo dos Campos, Federal University of Bahia, Brazil. This work was performed according to the guidelines of the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal, COBEA) and approved by the Ethics and Animal Welfare Committee (Comissão de Ética e Bem-Estar Animal, CEBEA) of the UFBA, Ondina campus Bahia.

2.1 Animals, experimental design and diets

Five uncastrated, 16 months old, crossbred (Dorper x Santa Ines) lambs with average body weight (BW) of 42.5 kg rumen cannulas were used in a 5 x 5 latin square design. The animals were housed in individual cages (1 x 1 m). The treatment diets consisted of levels of licuri inclusion to the diets as follow: 1) 0%; 2) 4%; 3) 8%; 4) 12% and 5) 16% of ground licuri (Table 1 and 2). The diets were formulated to be isonitrogenic and nitroenergetics according to the (NRC, 2007). The diets were composed of Tifton-85 (*Cynodon spp.*) hay, sisal (*Agave sislana*) silage byproducts and concentrate in the proportions 26, 28 and 47%, recpectively, with 53:47 of forage: concentrate ratio. (Table. 1).

2.2 Experimental and analytical procedures

The initial and final live weights were taken for each period. The feeding trial was carried out for five periods of 16 days each, with 10 days of adaptation to the respective diets, followed by 5 days of sample collection for the total mixed ration sample (TMR).

During the collection period, samples of TMR, orts and total feces were weighed daily, mixed, and a composite sample was taken, dried at 55 ° C and ground to a 1 mm screen pore size (Moinho Willye TE-680, TECNAL, Brazil) and stored for further analysis. The samples of TMR, ingredients, orts and feces were analyzed, according to AOAC (2010), for dry matter (method 934.01), ash (method 942.05), nitrogen (method

2001.11) and ether extract (EE; method 920.39). The NDF and ADF were analyzed, according to Van Soest et al., (1991) using heat stable amylase and sodium sulfite.

Table 10. Nutrient composition, particle distribution, physical effectiveness factor (pef), and physically effective fiber (peNDF) of dietary ingredients

Item	Ingredients				
	Tifton hay	Sisal silage by-products	Ground corn	Soybean meal	Ground licuri
Nutrients[1]					
DM ($g.kg^{-1}$ fresh weight)	912	209	874	874	914
Organic matter	940	893	990	933	976
Mineral matter	60	107	10	67	24
Crude protein	58	71	81	545	60
Ether extract	10	17	13	19	89
Neutral detergent fibre	744	288	181	217	672
Neutral detergent acid	348	160	22	120	393
Cellulose	249	176	29	66	188
Hemicellulose	316	110	56	34	237
Lignin	44	93	22	22	165
Nonstructural carbohydrates	128	517	625	151	155
Particle size distribution[2]					
19 mm	67.56	5.94	0.00	0.00	0.00
8 mm	11.71	9.54	0.00	0.00	29.18
1.18 mm	13.71	65.52	54.31	56.08	61.97
Pan	7.02	19.00	45.69	43.92	8.85
$pef_{8.0}$	0.79	0.15	0.00	0.00	0.29
$pef_{1.18}$	0.93	0.81	0.54	0.56	0.91
$peNDF_{8.0}$, % of DM	58.53	4.41	0.00	0.00	19.62
$peNDF_{1.18}$, % of DM	68.65	22.21	6.15	9.94	61.29

[1]Expressed as $g.kg^{-1}$ DM. [2]Particle size distribution of ingredients was measured using the Penn State Particle Separator (Kononoff et al., 2003); $pef_{8.0}$ and $pef_{1.18}$ = physical effectiveness factor determined as the proportion of particles retained on 2 sieves (Lammers et al., 1996) and on 3 sieves (Kononoff et al., 2003), respectively; $peNDF_{8.0}$ and $peNDF_{1.18}$ = physically effective NDF determined as NDF content of the diet multiplied by $pef_{8.0}$ and $pef_{1.18}$, respectively.

2.3 Intake and digestibility

The lambs were fed twice daily, at 8:00 h and 16:00 h, ensuring a 10% surplus and water supply was *ad libitum*. Individual intakes were recorded daily. 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. The chemical composition of the consumed feed was estimated using the ratio of the consumption of each nutrient to the intake of DM × 100. The non-fiber carbohydrates (NFC) were calculated according to Hall (2000), where:

NFC = 100 - [(% CP - % CP urea + % urea) + % EE + % ash + % NDF)], in which CP= Crude protein; EE = Ether extract; NDF = neutral detergent fiber.

The total digestible nutrients (TDN) of diets were determined by Sniffen et al. (1992):

TDN = DCP + 2.25*DEE + TDCH

Where: DCP = digestible CP; DEE = digestible EE; and TDCH = total digestible carbohydrates.

The TDN intake (TDNi) was calculated, according to Sniffen et al. (1992) using the equation:

TDNi = (CPi − CPf) + 2.25 (EEi − EEf) + TCi − TCf)

Where: CPi, EEi, and TCi represent the intake of CP, EE, and total carbohydrates, respectively, and where CPf, EEf, and TCf refer to the excretion of CP, EE, and total carbohydrate in the feces, respectively.

The coeficients of apparent digestibility (CD) of DM, OM, CP, EE, NDF, NSC were calculated using the following equation:

CD (%) = [(nutrient intake (g) - nutrient excretion (g)) / nutrient intake (g)] x 100.

The TDN content was calculated by the equation Sniffen et al. (1992):

TDN = intake of TDN/intake of DM

2.4 Nitrogen balance and microbial protein synthesis

In the same collection period (10 to 15 days), 10 ml urine spot samples per day were collected at different times (-2, 0, 2, 4, 6 and 8 hours) and mixed with 40 ml of 0.036 N H_2SO_4, a composite sample (50 ml) were taken and frozen. The nitrogen samples were determined using the kjeldahl method (AOAC, 1990). The nitrogen balance (NB) was calculated as the difference between the total nitrogen intake and the total nitrogen excreted in feces and urine:

$$NB\ (g.day^{-1}) = N_{intake} - (N_{feces} + N_{urinary}).$$

Table 11. Ingredients, nutrient composition, particle distribution, physical effectiveness factor (pef), and physically effective fiber (peNDF) contents of experimental diets

Item	Treatments[1]				
	0	4	8	12	16
Ingredients[2]					
Ground licuri	0	39	75	112	149
Vitamin/mineral mix	8	8	8	8	8
Soybean meal	122	127	124	126	126
Ground corn	323	295	247	208	169
Urea/ammonium sulphate	8	8	8	8	8
Tifton hay	258	264	256	254	258
Sisal silage by-products	281	258	282	284	281
Nutrients[2]					
DM ($g.kg^{-1}$ Fresh weight)	672	691	675	674	678
Organic matter	939	939	938	937	937
Mineral matter	61	61	62	63	63
Crude protein	151	153	149	148	147
Ether extract	20	22	24	26	29
Neutral detergent fibre	326	347	367	386	409
Neutral detergent acid	157	170	184	198	213
Cellulose	131	135	143	149	155
Hemicellulose	135	142	148	154	161
Lignin	47	51	58	63	69
NFC[3]	456	431	412	390	366
Particle size distribution[4]					
% of DM retained on sieves					
19.0-mm	19.64	19.86	19.51	19.39	19.64
8.0-mm	5.62	6.41	7.42	8.32	9.23
1.18-mm	45.81	45.69	46.67	47.19	47.31
Pan	29.56	28.32	26.99	25.72	22.09
$pef_{8.0}$	0.25	0.26	0.27	0.28	0.29
$pef_{1.18}$	0.71	0.72	0.74	0.75	0.76
$peNDF_{8.0,}$ % of DM	8.23	9.13	9.87	10.70	11.81
$peNDF_{1.18}$, % of DM	23.15	25.00	26.98	28.93	31.16

[1]Percentage inclusion of ground licuri in the diets. [2]Expressed as $g.kg^{-1}$ DM. [3]Non-fiber carbohydrates. [4]Particle size distribution of mixed diets was measured using the Penn State Particle Separator (Kononoff et al., 2003); $pef_{8.0}$ and $pef_{1.18}$ = physical effectiveness factor determined as the proportion of particles retained on 2 sieves (Lammers et al., 1996) and on 3 sieves (Kononoff et al., 2003), respectively; $peNDF_{8.0}$ and $peNDF_{1.18}$ = physically effective NDF determined as NDF content of the diet multiplied by $pef_{8.0}$ and $pef_{1.18}$, respectively.

The concentrations of creatinine (colorimetric method endpoint - Labtest, ref 35) and uric acid (colorimetric enzymatic method –Bioclin K139) in the urine were determined. Creatinine was used as a marker to estimate urinary volume, considering that the mean excretion of creatinine per lamb is 23.1 mg creatinine/BW (FAICHNEY et al., 1995). The urinary volume (UV) was estimated by UV (L) = BW * 23.1 mg/kg/creatinine in the urine (mg/L).

The urine allantoin, xanthine and hypoxanthine contents were estimated by colorimetric methods and the total purine derivatives (TP) excretion was calculated by the sum of hypoxanthine, xanthine, acid uric and allantoin in the urine and expressed as mmol/d. The absorbed purines (AP) were estimated based on the total amount of purine derivatives excreted in sheep (PE_{sheep}), according to Chen and Gomes, (1992):

$$PE_{sheep} = 0.84\ AP + (0.150\ MBW^{0.75} * e^{-0.25AP})$$

Where: PE_{sheep}= Purine derivatives excreted in sheep (mmol/d), AP= absorbed purines (mmol/d), MBW= metabolic body-weight (kg).
The supply of microbial nitrogen (MN) was estimated as follows:

$$MN\ (g.day^{-1}) = (70 * AP) / (0.83 * 0.116 * 1000)$$

Assuming that the digestibility of microbial purine is 0.83, the N content of purines is 70 mg N/mmol and the purine-N:total-N ratio in mixed rumen microbes is 11.6:100 (CHEN and GOMES, 1992).

2.5 Rumen content sampling and rumen pH

At the end of each period (16 days), 100 ml strained of ruminal fluid samples were collected at 0, 2, 4, 6 and 8 hours after feeding. Immediately after each collection, the pH was recorded (General Tools PH501 Digital); an aliquot subsample (50 ml) was taken and mixed with 1 ml of sulfuric acid (1:1 v/v) and frozen at -10 °C for ammonia analysis. The ammonia concentration was measured by colorimetric method, according to Chaney e Marbach (1962). The remaining 50 ml was analyzed for volatile fatty acids VFA (HPLC, Shimadzu SPD-10A VP coupled to UV detection-210 nm). At the 4 hours after

feeding from the same day (16 day) of collection of ruminal fluid, an additional 40 ml aliquot of ruminal liquid samples was retained for protozoal counts and mixed (1:1v/v) with 50% formalin. The density of rumen protozoa per milliliter was obtained by using a Sedgewick-Rafter counting chamber (DEHORITY, 1984) with the modifications proposed by D'agosto e Carneiro (1999). All ruminal samples were kept frozen before analysis.

2.6 Blood metabolites

On the last day of each experimental period (16 days), blood samples were taken from the jugular vein at 0 and 4 h after feeding using vacutainer tubes containing EDTA (Ethylene Diaminotetraacetic Acid), the collected blood samples were centrifuged at 1,600 rpm for 10 minutes and the serum was isolated and stored frozen until analyzed.

The serum samples were then quantified in a spectrophotometer (Bio-Rad Laboratories, Hercules, USA) at length specified in each procedures. The blood serum was assessed for following parameters: Triglycerides, urea, glucose, albumin, creatinine (Labtest® Diagnóstica S.A., Salvador, BA, Brazil), aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Doles® Reagentes Brazil) and Non-esterified fatty acids (NEFA) (Randox®).

2.7 Statistical analysis

The data were analyzed using the PROC MIXED of SAS in a 5x5 Latin Square design, with animal and period as random effect and licuri addition as fixed effect of the model. Polinomial contrasts were used to test the linear and quadratic effects of licuri supplementation on all parameters. The temporal effect of the diets on blood parameters after feeding was analyzed in a repeated measure design. Before the analyses, the best error structure was defined based on the AIC parameters using the command REPEATED. Degrees of freedom and tests were adjusted using the Kenward-Roger option. The time effect was decomposed in 2 orthogonal polynomial contrasts: linear, quadratic. Significance was declared at $P < 0.05$.

3.0 RESULTS

3.1 Intake and digestibility

The inclusion of licuri in the diets did not affect (P>0.05) the intake and digestibility of DM, OM, CP, EE, NFC and TDN (Table 3).

Table 12. Least square means of nutrients intake and digestibility of lambs fed increasing levels of ground licuri

Item	Treatments[1]					SEM[3]	P[2]	
	0	4	8	12	16		L	Q
Intake (g kg^{-1})								
Dry matter	1156.1	1108.3	990.8	1092.8	1022.3	100.5	0.485	0.364
Organic matter	1080.5	1080.7	909.1	1021.0	909.1	88.90	0.375	0.203
Crude protein	158.7	151.9	135.3	141.9	138.9	18.43	0.375	0.322
Ether extract	28.0	27.8	24.3	24.4	27.4	3.491	0.365	0.334
NDF[4]	444.3	382.3	416.7	429.1	443.8	45.48	0.943	0.793
NFC[5]	419.3	447.2	348.1	306.1	306.1	42.39	0.164	0.054
TDN[6]	840.6	781.9	781.8	836.1	887.3	29.23	0.916	0.939
Digestibility coefficients								
Dry matter	0.723	0.714	0.767	0.728	0.668	0.029	0.566	0.319
Organic matter	0.756	0.746	0.794	0.757	0.670	0.032	0.731	0.451
Crude protein	0.723	0.717	0.774	0.724	0.683	0.040	0.717	0.448
Ether extract	0.714	0.632	0.751	0.705	0.686	0.039	0.591	0.186
NDF[4]	0.681	0.634	0.738	0.667	0.604	0.045	0.747	0.328
NFC[5]	0.856	0.880	0.863	0.854	0.755	0.039	0.835	0.711
TDN[6]	0.729	0.717	0.764	0.724	0.644	0.003	0.799	0.473

[1]Percentage inclusion of ground licuri seed. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects. [3]SEM standard error of the mean. [4]Neutral detergent neutral. [5]Non-fibrous carbohydrates [5]Total digestible nutrients.

3.2 Nitrogen balance and microbial protein synthesis

The inclusion of licuri in the diets did not affect (*P*>0.05) the nitrogen balance, the nitrogen in feces, absorbed, urinary, and protozoa count (Table 5). In the same way, the inclusion of licuri did not affect (*P*>0.05) the microbial N and microbial efficiency (Table 5).

Table 13. Least square means of nitrogen balance, microbial N, microbial efficiency, and numbers protozoa in ruminal fluid of lambs fed increasing levels of ground licuri

Item	Treatments[1]					SEM[3]	P[2]	
	0	4	8	12	16		L	Q
Nitrogen balance (g day^{-1})								
N intake	21.65	24.86	24.42	26.88	24.88	1.984	0.104	0.847
N feces	4.85	7.01	5.65	6.56	7.19	0.782	0.244	0.377
N urinary	8.16	9.06	6.88	8.64	10.07	0.824	0.804	0.513
N absorbed	16.39	17.36	18.74	20.02	17.75	1.556	0.094	0.913
N retained g.day^{-1}	8.09	7.98	11.46	11.55	8.22	2.540	0.244	0.968
MBW[3]	1.04	1.12	1.10	1.24	1.06	0.142	0.349	0.844
N retained /intake^{-1}	35.74	31.35	46.48	42.07	30.50	6,574	0.266	0.999
Microbial N	14.02	10.98	10.76	11.91	13.56	3.223	0.644	0.514
Microbial efficiency[5]	103.8	88.47	83.12	90.26	96.47	23.84	0.662	0.634
Protozoa (log10) cells/mL	5.20	5.75	5.16	5.61	5.59	0.240	0.555	0.728

[1]Percentage inclusion of ground licuri. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects. [3]Standard error of the mean. [4]Metabolic body weight. [5]Grams of microbial protein.kg^{-1} TDN.

3.3 Ruminal parameters

Including licuri in the diets did not affect the pH and the ruminal concentration of NH_3-N, acetate, propionate, butyrate, lactic acid, acetate:propionate ratio and total VFA among treatments (Table 4). However, the data showed a quadratic effect (P>0.05) for collection times (0, 2, 4, 6 and 8 hours after feedeing) within pH and ruminal

Table 14. Least square means of ruminal fluid pH, NH_3-N, and VFA concentrations of lambs fed increasing levels of ground licuri

Item	Treatments[1]					SEM[3]	*P*[2]				
							Treat		Time		Treat x Time
	0	4	8	12	16		L	Q	L	Q	
pH	6.36	6.54	6.56	6.54	6.63	0.090	0.130	0.188	<0.001	<0.001	0.965
NH_3-N (mg/dL)	11.74	14.38	12.17	13.43	14.77	2.024	0.734	0.709	<0.001	<0.001	0.560
Total VFA m*M* /L	65.27	60.22	67.60	60.28	64.92	10.099	0.833	0.887	<0.001	0.007	0.655
Acetate	50.30	46.88	52.63	49.01	49.63	7.918	0.949	0.988	<0.001	0.041	0.679
Propionate	10.34	10.94	9.32	9.68	9.28	1.879	0.661	0.946	<0.001	<0.001	0.507
Butyrate	5.34	3.80	4.17	3.07	3.76	1.108	0.043	0.728	<0.001	0.046	0.826
Acetate: propionate	5.82	5.83	12.41	6.63	6.36	2.770	0.470	0.301	0.218	0.129	0.378

[1]Percentage inclusion of ground licuri seed. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects for treatment (Treat), collection time (Time) and interaction between treatments and time (Treat x Time). [3]SEM standard error of the mean.

concentration of NH_3-N, acetate, propionate, butyrate, and total VFA (Figures 1 to 7). There was no intereaction effect between treatments and collection time (Table 4).

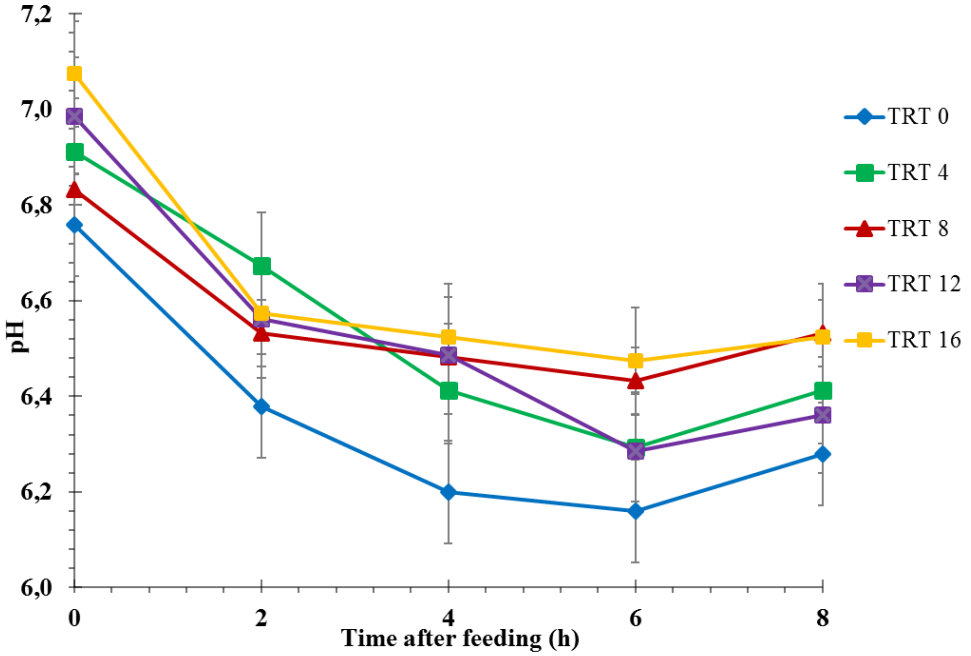

Figure 4. Temporal change of pH in the ruminal fluid of lambs fed ground licuri. TRT; Percentage inclusion of ground licuri

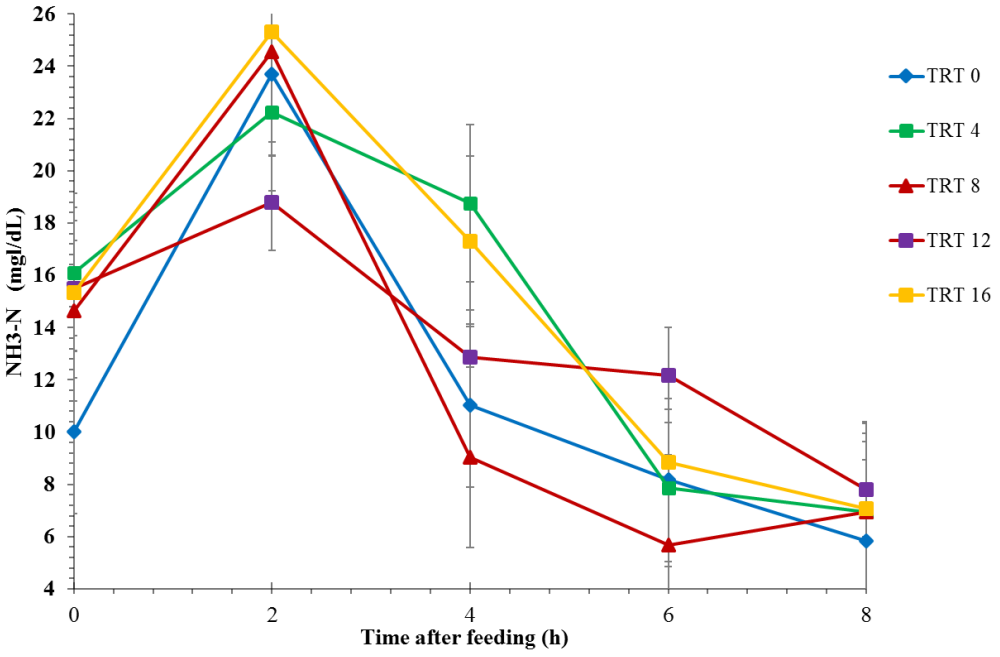

Figure 5. Temporal change of NH3-N (mg/dL) in the ruminal fluid of lambs fed ground licuri. TRT; Percentage inclusion of ground licuri

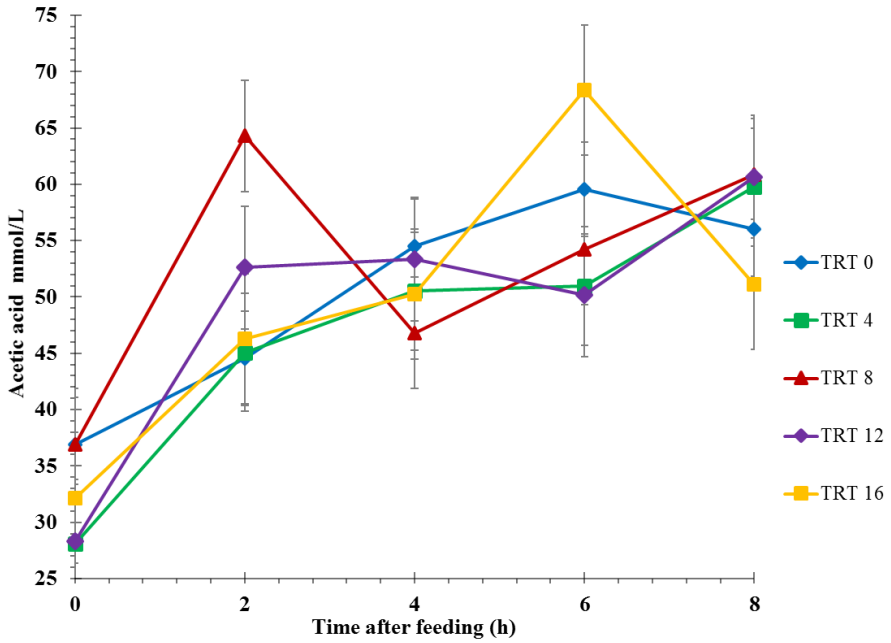

Figure 6. Temporal change of acetic acid in the ruminal fluid of lambs fed ground licuri. TRT; Percentage inclusion of ground licuri

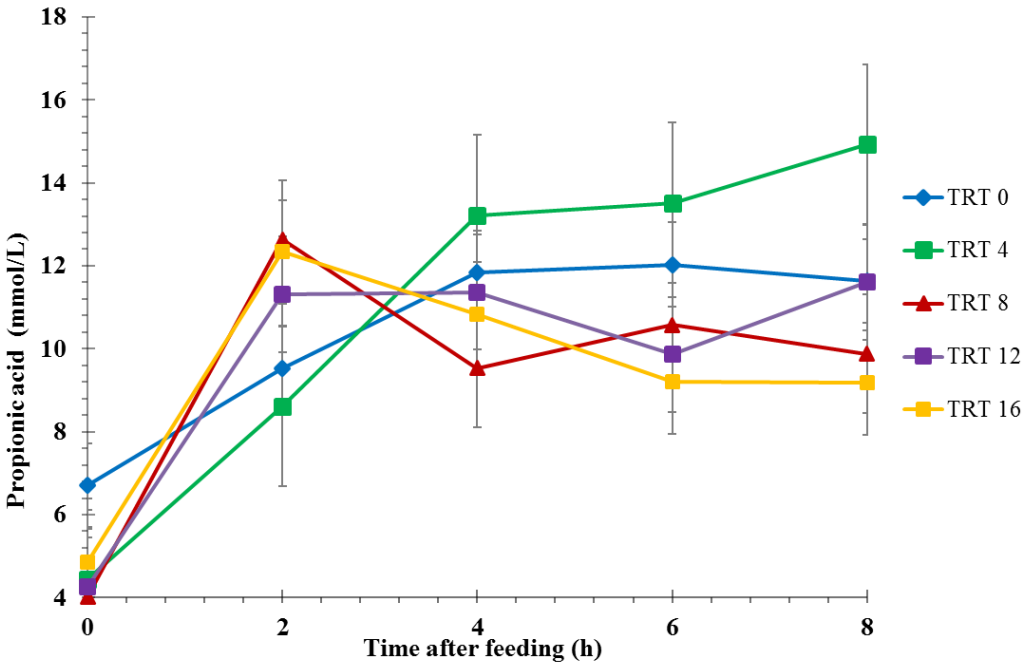

Figure 7. Temporal change of propionic acid in the ruminal fluid of lambs fed ground licuri. TRT; Percentage inclusion of ground licuri

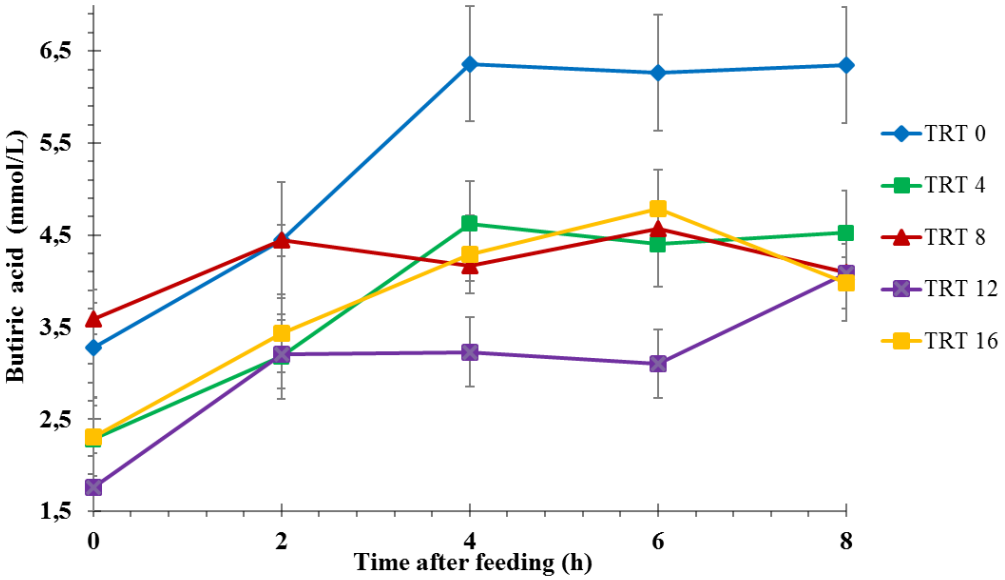

Figure 8. Temporal change of butyric acid in the ruminal fluid of lambs fed ground licuri. TRT; Percentage inclusion of ground licuri

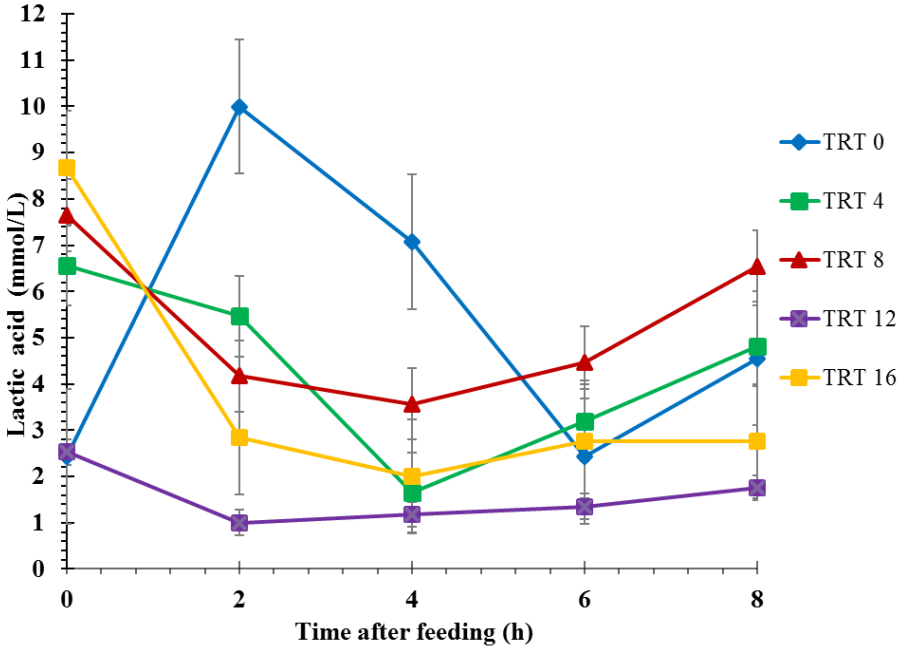

Figure 9. Temporal change of lactic acid in the ruminal fluid of lambs fed ground licuri. TRT; Percentage inclusion of ground licuri

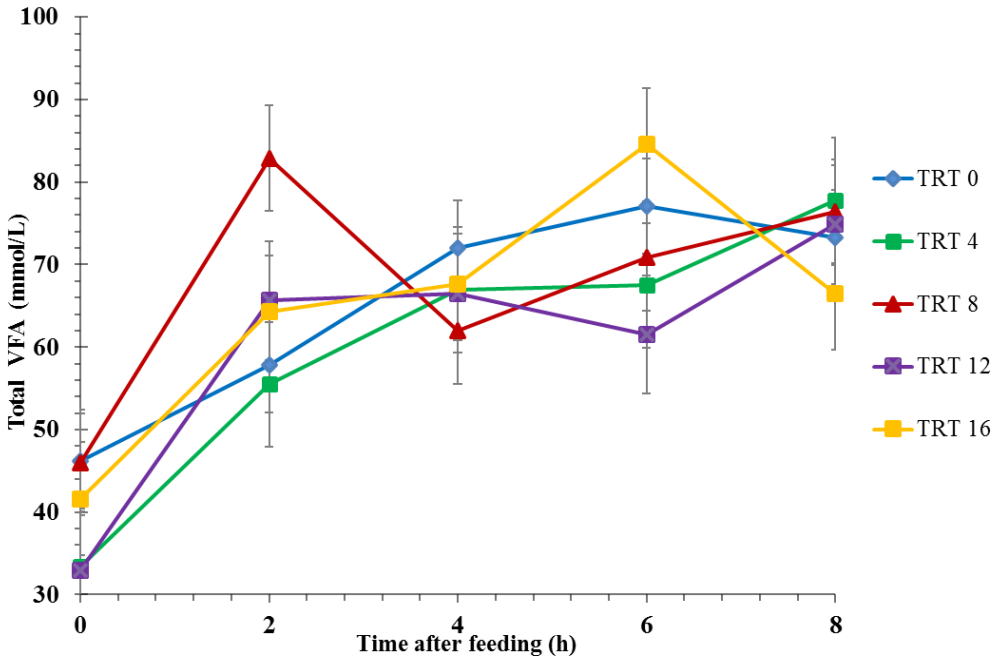

Figure 10. Temporal change of total VFA in the ruminal fluid of lambs fed ground licuri. TRT; Percentage inclusion of ground licury seed.

3.4 Blood metabolites

Inclusion of licuri in diets did not affect (P>0.05) the serum concentration of creatinine, urea, albumin, triglycerides, non-esterified fatty acids (NEFA), and glucose between treatments (Table 6).

Table 15. Least square means of blood parameters of lambs fed increasing levels of ground licuri

Item	Treatments[1]					SEM[3]	P[2]	
	0	4	8	12	16		L	Q
Creatinine (mg/dL)	1.04	0.74	1.10	1.02	0.78	0,145	0.604	0.464
Urea (mg/dL)	43.21	46.27	46.96	49.36	49.75	3,938	0.284	0.934
Albumin (g/L)	36.94	38.00	33.65	36.27	36.59	1,510	0.095	0.328
Triglyceride (mg/dL)	87.95	89.51	87.51	88.51	89.48	4,189	0.975	0.976
NEFA[4] mmol/L	1.61	1.59	1.91	1.62	1.59	0,131	0.475	0.297
Glucose (mg/dL)	86.55	91.22	88.97	97.18	94.01	2,798	0.072	0.520

[1]Percentage inclusion of ground licuri seed. [2]Probabilities of orthogonal contrasts for testing linear (L) and quadratic (Q) effects of the treatments. [3]Standard error of the mean. [4] Non-esterified fatty acids.

4.0 DISCUSION

The diets were formulated to be isonitrogenous and isoenergetic (Table 2). However, the inclusion of licuri markedly increased the NDF, peNDF$_{>8.0}$, peNDF$_{>1.18}$ and EE in in the diets (14.6, 34.7, 23.4 and 36.0 %, respectively) and, consequently, decreased NFC (17.2%) concentration.

We expected that the increase of NDF and peNDF in the diets would decrease the intake, because higher NDF and peNDF often limits voluntary feed intake (MERTENS, 1991; OBA and ALLEN, 1999), but this did not occur. However, using ground alfalfa hay in goats, increasing forage particle length increased intake of peNDF, but decreased DMI linearly (ZHAO et al., 2010). Similarly, using barley silage, increasing forage particle length in the diets of dairy cows increased intake of peNDF; although, the digestibilities of NDF in the total tract was linearly decreased with increasing dietary peNDF (YANG and BEAUCHEMIN, 2006b). Contrary, diets varied in peNDF content (high, medium, and low) by altering the particle length of corn silage showed that increasing peNDF content of diets improved fiber digestion (YANG and BEAUCHEMIN, 2006a), improved total digestibility (KONONOFF and HEINRICHS, 2003).

The increase of EE in ruminant rations increases energy density of the diets and high EE in diets often decrease DMI, depending on the FA profile (CHOI and PALMQUIST, 1996; RELLING et al., 2010). We expected that the increase of EE in the diet would increase it intake and digestibility (NRC, 2001). However, this effect was not observed in this study. The inclusion of licuri did not affect (P>0.05) the variables of intake and digestibility, although showed the decrease in the DMI (11.6 % non-significant) that resulted in intake similar of NDF and EE (Table 3), despite of increase of NDF and EE in the diets.

Moreover, there was no effect of licuri addition on nutrient digestibility, despite that fat may inhibit fiber digestion in the rumen (PALMQUIST and JENKINS, 1980). The lack of response on intake and digestibility may be partially explained by animal selection of feed ingredients. Also, the EE in the diets were less than 50 g/kg, which is considered a low amount of fat to be able to negatively affect intake and digestibility of dietary nutrients (PALMQUIST, 1976).

The pH and the concentration of NH_3-N in the rumen fluid were not affected by licuri inclusion, but they increased 4.2% and 25.8%, repectivelly, form the lowest to the highest amount of licuri in the diets. Likewise, the highest concentration of ammonia agrees with the highest ruminal pH in the diet with 16 % of inclusion of ground licuri. The concentration of ammonia depends mainly on pH; and supplemental fat does not modify rumen pH in most experiments (DOREAU; FERLAY, 1995). The increase the peNDF showed beneficial effects of increasing chewing activity and rumen pH on dairy cows (YANG and BEAUCHEMIN, 2005), and goats (ZHAO et al., 2010).

The lipid supplementation generally resulted in either a decrease or, more often, no variation in ammonia concentration in the rumen (DOREAU; FERLAY, 1995). The total VFA, acetate, propionate, butyrate and acetate:propionate ratio showed no effect. However, higher pH corresponded to lower concentration of propionate and, thus, a higher acetate:propionate ratio that decreased by 10.3 %, althought not significant). These non significant changes in the pH and propionate concentration could be attributed to the lower NFC and higher peNDF of diets. Contrary to this study, Zebeli et al. (2006) reported that total VFA was negatively affected by increasing dietary peNDF, what might be due to the positive effect of peNDF on rumen motility. Likewise, Yang and Beauchemin (2005) described that the influence of dietary peNDF on ruminal pH and fermentation was minimal. The pH, NH3-N, total VFA, acetate, propionate and butyrate on collect time showed a quadratic effect ($P<0.05$), this expected response to the digestive process.

We can not explain the quadratic effect ($P>0.05$) of lactic acid, which is mainly associated with NFC that gradually decrease with the inclusion of licuri (Table 4). Despite of the increase de NH3-N in the rumen (Table 4), similar intake of TDN and lower NFC intake among treatments, the nitrogen balance, microbial N, and microbial efficiency showed no effect (Table 5). The average NH3-N among treatments (13.3 $mg.dL^{-1}$) was well above the 5 mg/dL minimum suggested for maximal bacterial CP synthesis (FIRKINS et al., 2007).

There were no interactions between treatment and collection time on all variables (Table 4). However, ruminal microbial protein and microbial efficiency depends on supply of adequate amounts and type of carbohydrate as an energy source for the synthesis of peptide bonds; therefore, readily fermentable carbohydrates such as starch or

sugars are more effective than other sources such as cellulose in promoting microbial growth (STERN; BACH; CALSAMIGLIA, 2006). The excess ammonia not used by bacteria is absorbed and converted to urea in the liver which reflects an increase (15% non-significant) of urea in the blood (Table 6). Likewise, creatinine, albumin, trygliceride, NEFA and glucose were not affected ($P>0.05$) by treatments (Table 6).

The increased EE with inclusion of licuri was associated to the MCFA. The licuri contain higher amounts of MCFA (80%), mainly lauric acid (42%) and myristic acid (15%) (BAUER et al., 2013). However, the ruminal protozoa population can decrease by feeding MCFA (REVENEAU et al., 2012). The lauric acid and sodium laurate were effective on reducing protozoa numbers ($P<0.01$) and had no effect on the DM intake and digestibility (FACIOLA et al., 2012; FACIOLA and BRODERICK, 2014). The lauric acid and myristic acid exert a synergistic action against ruminal protozoa (SOLIVA et al., 2003). The efficiency of microbial protein synthesis was increased and protozoa concentrations tended to decrease when fat was fed (OLDICK; FIRKINS, 2000). Hristov and Jouany (2005) described that the palatability and reduced DMI are the major factors leading to decreased microbial protein synthesis with MCFA or coconut oil.

However, these observations did not occur with the licui addition. There was no difference on the protozoa population, microbial protein synthesis and microbial efficiency (Table 5), probably because the concentration of MCFA were lower than that used in the experiments cited above; also, the form of the fatty acids supplied (ground licuri) used requires more time for the availability of fatty acids in the rumen when compared to the others experiments. A direct oil addition, instead of ground or crushed oilseeds has a more pronounced and negative effect on ruminal ecosystem (HOFFMANN et al., 2013).

5.0 CONCLUSION

The ground licuri can be included in the lambs diet up to 16% in substitution of ground corn. The increase of NDF and EE by the inclusion of ground licuri did not affect the rumen parameters, microbial protein synthesis, microbial protein synthesis efficiency and blood parameters.

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IMPLICATIONS

We carried out two experiments with ground licuri addition to lamb diets. In Experiment 1, we evaluated the intake, digestibility and MPS using 40 growing lambs in a completely randomized design. In Experiment 2, we evaluated intake, digestibility, MPS and ruminal metabolism using 5 adult lambs in a 5 x 5 Latin square design. The inclusion of ground licuri increased NDF, peNDF$_{> 8}$, peNDF$_{> 1.18}$, and EE in both experiments; however, the lack of effect on experiment 2 can be explained by the differences in the relation V:C of experiment 1 (V:C 46:54) and experiment 2 (V:C 53:47).

On the other hand, it is well known that the northeastern region of Brazil has a low production of forage, therefore, it is possible to use ground licuri on feeding sheep because the carcass quality was not altered even though the yields achieved in this study they decrease from 215 grams per day to 154 grams per day (15% inclusion).