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# Creatinine and purine derivatives excretion and microbial synthesis in lambs fed rain tree pod meal

[*Excreção de creatinina e derivados de purina e síntese microbiana em cordeiros alimentados com farinha de vagem de* Samanea saman]

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## ABSTRACT

Evaluated the levels of rain tree (*Samanea saman*) pod meal (RTPM) (0, 10, 15, 20 and 25%) replacing maize in the dry matter of the diet on intake of DM, CP and ME, creatinine and total purine derivatives excretion in urine and microbial protein synthesis in lambs. Twenty-five uncastrated Bergamasca lambs were used, with an initial body weight of 24±5kg and an average age of 120 days. The experimental design was completely randomized, with five treatments and five replications. The trial lasted 84 days and the 24h and *spot* urine collections were performed in the last day of the experiment. The intake was not affected (P>0.05), the urine volume and daily creatinine excretion were influenced (P<0.05) by RTPM replacing maize, observing increasing linear effect and cubic variation (P<0.05), respectively. The cubic and quadratic components were significant (P<0.05) for the excretion of total purine derivatives, absorbed purines, and microbial synthesis. The cubic variation, with peaks at 5% and 18% replacement for urinary excretion of creatinine and purine derivatives, indicates that the levels used of RTPM affected the renal activity of lambs. Substitution of maize by RTPM up to 10% enhances efficiency of rumen microbial crude protein synthesis.

Keywords: Fabaceae, Kidney, Phytochemicals, Rain tree, *Samanea saman* (Jacq.) Merr

## RESUMO

*Avaliaram-se os níveis de farinha de vagem de* Samanea saman *(0, 10, 15, 20 e 25%) em substituição ao milho na matéria seca da dieta sobre o consumo de MS, PB e EM, a excreção urinária de creatinina e de derivados de purina totais e a síntese de proteína microbiana em cordeiros. Foram utilizados 25 cordeiros Bergamácia, não castrados, peso corporal inicial de 24±5kg e idade média de 120 dias. O delineamento experimental foi inteiramente ao acaso, com cinco tratamentos e cinco repetições. O experimento durou 84 dias e as coletas de urina* spot *e de 24h foram realizadas no último dia do experimento. O consumo não foi afetado (P>0,05), o volume de urina e a excreção diária de creatinina foram influenciados (P<0,05) por* S. Saman *substituindo o milho, observando-se efeito linear crescente e variação cúbica (P<0,05), respectivamente. Os componentes cúbicos e quadráticos foram significativos (P<0,05) para a excreção de derivados de purina totais, purinas absorvidas e síntese microbiana. A variação cúbica, com picos em 5% e 18% de substituição para a excreção urinária de creatinina e de derivados de purina, indica que os níveis utilizados de* S. saman *afetaram a atividade renal de cordeiros. Substituição do milho por farelo de vagem de* S. saman *até 10% aumenta a eficiência de síntese de proteína bruta microbiana.*

*Palavras-chave: Depuração renal, Fabaceae, Fitoquímicos,* Samanea saman *(Jacq.) Merr*

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## INTRODUCTION

The Brazilian herd of sheep totaled 19.715 million head, mostly distributed in the Northeast, accounting for 68.5% of the actual number of heads (Pesquisa…, 2019). Nevertheless, this potential does not translate into higher production of sheep meat. It is known that lower production is partly because sheep farming still presents technique weakness.

One of the biggest obstacles to sheep production is feeding and it represents the largest portion of the production cost. Conventional foods such as maize and soybean are the main sources of energy and protein for animal feeding. These are products commercialized in the international market suffering price changes according to the international offering, which can derail sustainable use in the sheep industry in Northeastern Brazil.

The occurrence of rain tree (*Samanea saman* Jacq. Merr.) covers the valley of the river San Francisco and can be promising due to annual production of pods that coincides with the dry season (Lorenzi, 1998). Moreover, these are palatable and consumed by cattle, sheep and goats, because pods present a fleshy and sweet pulp (Delgado *et al*., 2014).

Despite *S. saman* pods providing desirable levels of nutrients, it also shows significant levels of secondary metabolic compounds that may be limiting the performance or damaging the health of the animal when consumed in excess, such as alkaloids, saponins, resins, steroids, glycosides, and tannins (Delgado *et al*., 2014). However, there is still limited information on these phytochemicals and their biological activities (Obasi *et al*., 2010). A study by Ukoha *et al*. (2011) demonstrated that these secondary metabolites have an action against various bacterial species. Thus, it could interfere with rumen microbial protein synthesis. The population of methanogens and protozoa decreased and population of *Fibrobacter succinogenes* increased, while *Ruminococcus flavefaciens* and *R. albus* were not affected with daily supplementation of saman pod at 60g $kg^{-1}$ of total dry matter intake in lactating dairy cows (Anantasook *et al*., 2015).

Many techniques used to measure the microbial nitrogen flow from the rumen to the small intestine require surgically prepared animal. Chen and Gomes (1992); Chen *et al*. (1992) proposed alternatives techniques less invasive for the estimation of microbial protein synthesis in the rumen using the excretion of purine derivatives in urine. For this, it is necessary to obtain the daily urine output, which can be estimated using spot urine samples, dividing the daily creatinine excretion by the concentration of creatinine in the spot urine.

The use of reference index for daily creatinine excretion is possible as an indicator to estimate the urine volume, since it presents constant renal excretion, independent of diet and proportionate to body mass. It allows obtaining the excretion of purine derivatives and other metabolites in a spot urine sample at 4h after morning feeding without the need for total collection of 24 h-urine (Chen and Gomes, 1992; Oliveira *et al*., 2001; Rennó *et al*., 2008; Pereira, 2015; Santos *et al*., 2017, 2018).

Depending on the dose given, the secondary metabolites from rain tree pods, such as pithecolobin alkaloid and saponin, have been implicated in altering renal activity (Rhiouani *et al*., 1999; Roshni *et al*., 2014). Thus, it is necessary to evaluate whether the incorporation of this unconventional foodstuff affects the daily excretion of creatinine compromising its use as a urinary volume marker. The objective was to evaluate levels of rain tree pod meal (RTPM) (0, 10, 15, 20 and 25%) replacing maize in the diet dry matter on the daily excretions of creatinine and purine derivatives in the urine using the total collection of 24 h-urine and spot urine sampling at 4 h after morning feeding for estimation of microbial protein synthesis in lambs.

## MATERIAL AND METHODS

All the animal care and handling procedures were approved by the Ethics Committee on Animal Use of the State University of Southwest Bahia – UESB, Itapetinga Campus, with protocol number 11/2012. The experiment was conducted at the Animal Reproduction sector of the State University of Southwest of the Bahia – UESB, Itapetinga Campus, located in the Southwest region of the State of Bahia, at 15°09’07” South latitude, 40°15’32” West longitude of Greenwich and average altitude of 268m. Annual average temperature and rainfall are, respectively, 27°C and 800mm.

Twenty-five uncastrated Bergamasca lambs were utilized with an average initial body weight of 24 ± 5 kg and an average age of 120 days, wormed with anthelmintic levamisole phosphate in dosages recommended by the manufacturer. The animals were taken to individual stalls with dimensions of 1.7 x 2.0m, cement floor lined with coffee hulls and individual access to food and water. The experimental design was completely randomized, with five treatments and five replications, each lamb being an experimental unit. After an adaptation period of 10 days to the stalls, handling and feeding, the experimental period of 84 days began. The lambs were weighed fasted at first day and end of the experiment period to calculate the average body weight.

We evaluated five treatments which constituted of levels of RTPM (0, 10, 15, 20 and 25%) replacing maize in diet dry matter. The isonitrogen diets constituted of 400g $kg^{-1}$ DM of Tifton 85 hay (3-5cm particle size) and 600g $kg^{-1}$ DM of concentrate, balanced for weight gain of 200g per day (Nutrient…, 2007). The diets were offered every day at 0730 and 1600, *ad libitum*, to allow 10% leftovers.

The daily voluntary intake was calculated as the difference between the total diet offered and the leftover. The amount of food provided and the leftovers were registered for each animal and samples of Tifton 85 hay, concentrate and leftovers of total mixed ration per animal were collected and placed in plastic bags and frozen (-20ºC) for later analysis.

Samples of hay, concentrate, leftovers and faeces were analysed for dry matter (DM, method 930.15), ash (method 942.05), crude protein (CP, method 984.13), ether extract (EE, method 920.39) according to AOAC (Official…, 2005). For neutral detergent fibre (NDF), samples were analysed with thermostable alpha-amylase without the use of sodium sulphite and corrected for residual ash (Mertens, 2002). The correction of NDF for nitrogen compounds and estimation of insoluble nitrogen content in neutral detergent (NDIP) was done according to Licitra *et al.* (1996). The lignin (method INCT-CA F005/1) was obtained by the method described in Detmann *et al.* (2012), with the ADF residue treated with 72% sulfuric acid. The concentration of non-fibre carbohydrates (NFC) was calculated by adapting the method proposed by Hall (2003), NDFap. The ingredients and the chemical composition of the diets are shown in Table 1 and 2.

The faeces of each lamb were collected for three consecutive days by morning and evening sampling, and three times throughout the experimental period for the determination of apparent digestibility. The samples were inserting in plastic bags and stored in a freezer at -20ºC for later analyses. The faecal excretion was estimated using neutral detergent indigestible fibre (iNDF) as an internal indicator (Casali *et al*., 2008). The digestibility coefficients of the nutrients were calculated as the ratio between the amount of nutrient digested and ingested, multiplied by 100. The total digestible nutrients (TDN) were calculated according to Weiss (1999), using NDF and NFC corrected for ash and protein. There was a quadratic variation for total digestible nutrients ($P = 0.0001$; regression equation: $\hat{Y} = 68.11 + 0.56X - 0.031X^2$, $R^2 = 0.96$) (Table 2).

Table 1. Composition in ingredients of the experimental diets

| Item | Replacing level (%DM) | | | | |
|---|---|---|---|---|---|
| | 0 | 10 | 15 | 20 | 25 |
| | Composition in ingredients (g $kg^{-1}$ DM) | | | | |
| Tifton 85 hay | 400 | 400 | 400 | 400 | 400 |
| Maize | 288 | 259 | 245 | 230 | 215 |
| Rain tree pod meal | 0 | 29 | 43 | 58 | 73 |
| Soybean meal | 300 | 300 | 300 | 300 | 300 |
| Urea | 5 | 5 | 5 | 5 | 5 |
| Sodium chloride | 2 | 2 | 2 | 2 | 2 |
| Mineral salt[a] | 5 | 5 | 5 | 5 | 5 |

[a] Composition: Sodium 147.00g; Calcium: 120.00g; Phosphorus: 87.00g; Iron: 1,800.00mg; Cobalt: 40.00mg; Chromium: 20.00mg.

Table 2. Chemical composition of the experimental diets

| Item | Replacing level (%DM) | | | | |
|---|---|---|---|---|---|
| | 0 | 10 | 15 | 20 | 25 |
| | Chemical composition | | | | |
| Dry matter (g $kg^{-1}$ NM) | 905 | 903 | 904 | 902 | 904 |
| Crude protein (g $kg^{-1}$ DM) | 245 | 239 | 237 | 242 | 250 |
| Ether extract (g $kg^{-1}$ DM) | 34 | 29 | 32 | 27 | 27 |
| Non-fibre carbohydrates (g $kg^{-1}$ DM) | 238 | 305 | 272 | 294 | 278 |
| Neutral detergent fibre (g $kg^{-1}$ DM) | 423 | 443 | 472 | 448 | 461 |
| NDIP (g $kg^{-1}$ DM)[a] | 56 | 68 | 69 | 57 | 55 |
| Total digestible nutrients (g $kg^{-1}$ DM) | 680 | 712 | 683 | 674 | 624 |
| Metabolisable energy (Mcal $kg^{-1}$ DM)[b] | 2,46 | 2,57 | 2,47 | 2,44 | 2,26 |
| Lignin (g $kg^{-1}$ DM) | 77 | 76 | 74 | 80 | 71 |

[a] Neutral detergent insoluble protein; [b] Estimated according to NRC (Nutrient…, 2001).

On the last day of the experiment, spot urine samples of each animal were collected 4h after morning feeding. The measurement of the total production of 24 h-urine was performed using accumulated collections of urine volume obtained by spontaneous urination and preserved in 100mL of 20% $H_2SO_4$. The daily creatinine excretion was obtained using the 24 h-urine sample to determinate the creatinine concentration and the total volume of urine measured on the same day. The daily creatinine excretion (mg $kg^{-1}$ BW per day) of each animal was obtained as (CC x UV)/BW. Where: CC = creatinine concentration (mg $L^{-1}$) in 24h-urine sample (total collection); UV = urine volume (L) obtained in 24h; BW = body weight (kg).

The spot urine sample from each animal collected at the time point 4h after morning feeding was used to estimate the urine volume. The estimated urine volume was calculated as the average daily creatinine excretion divided by the concentration in the spot urine sample.

Aliquots of 10mL of urine samples collected at 4h were diluted in 40mL of 0.018 M $H_2SO_4$. These samples were prepared at pH below 3.0 to prevent bacterial destruction of the metabolites present in urine and immediately after, samples were stored at -20°C and destined for quantification of concentrations of creatinine, allantoin, uric acid, xanthine and hypoxanthine. The uric acid and creatinine concentrations in urine were determined using commercial kits (Bioclin®). Urinary concentrations of allantoin, hypoxanthine and xanthine were determined by spectrophotometric methods according to Chen and Gomes (1992).

The total excretion of purine derivatives was calculated by summing the quantities of allantoin, uric acid, xanthine and hypoxanthine in urine expressed in mmol $day^{-1}$. The amount of absorbed microbial purine (mmol $day^{-1}$) was estimated from the total excretion of purine derivatives (mmol $day^{-1}$) using the equation proposed by Chen and Gomes (1992) for sheep.

The intestinal flow of microbial nitrogen (g $day^{-1}$) was estimated from the absorbed purines (mmol $day^{-1}$) according to the equation described by Chen and Gomes (1992). The microbial synthesis efficiency was obtained by dividing the microbial protein synthesis (g $day^{-1}$) with the intake of total digestible nutrients (kg $day^{-1}$).

Intake, TDN concentration, urine volume and daily creatinine excretion obtained by total collecting of 24 h-urine and purine derivatives excretion, microbial synthesis and microbial efficiency obtained by spot urine collection were subjected to variance analysis using the statistical program Mixed Procedure (PROC MIXED) from the statistical package of SAS (Statistical…, 2006).

The polynomial contrasts were performed for the levels of RTPM (0, 10, 15, 20 and 25%) replacing maize in the diets. The following statistical model was adopted:

$Yij_k = (\beta_0 + \beta_1 Tr + \beta_2 Tr^2 + \beta_3 Tr^3) + \varepsilon ij_k$; NID $(0; \sigma^2)$, where Y = the estimated value according to the diets; $\beta_0$ = intercept; $\beta_1$; $\beta_2$ and $\beta_3$ defined the variation of Y according to the level of substitution; Tr = level of substitution (0, 10, 15, 20 and 25% of RTPM). For the linear, quadratic and cubic effects of diet regression analysis was performed. For all statistical procedures, the critical level adopted was 5% probability for Type I error.

## RESULTS

The substitution levels of maize with RTPM in the diets did not affect (P>0.05) the intake of DM, CP, and ME, which showed daily average values of 1.13kg, 0.30kg and 2.76Mcal (Table 3).

The urine volume increased (P<0.05) with 15mL increments for each RTPM percentage unit replacing maize in the diet. The quadratic and cubic components of the effect of RTPM levels in diets were significant (P<0.05) for daily creatinine excretion, adjusting cubic equations in the regression analysis (Table 3). The minimum and maximum levels of RTPM replacing maize in diets that provided the highest creatinine excretion were estimated at around 5% and 18%, respectively.

Table 3. Intake of dry matter (DM), crude protein (CP) and metabolizable energy (ME), urine volume and daily creatinine excretion in lambs fed rain tree pod meal levels replacing maize

| Item | Replacing level (%DM) | | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 15 | 20 | 25 | SEM | L | Q | C |
| Intake | | | | | | | | | |
| DM (kg day$^{-1}$) | 1.10 | 1.13 | 1.18 | 1.02 | 1.22 | 0.71 | 0.446 | 0.321 | 0.287 |
| CP (kg day$^{-1}$) | 0.30 | 0.29 | 0.30 | 0.27 | 0.33 | 0.94 | 0.369 | 0.289 | 0.134 |
| ME (Mcal day$^{-1}$) | 2.71 | 2.90 | 2.95 | 2.50 | 2.76 | 1.46 | 0.169 | 0.233 | 0.238 |
| Urine volume (L) | 0.66 | 0.72 | 0.94 | 0.60 | 1.21 | 0.07 | 0.003[a] | 0.283 | 0.090 |
| Creatinine | | | | | | | | | |
| mmol kg$^{-1}$ BW | 0.13 | 0.15 | 0.10 | 0.10 | 0.29 | 0.09 | 0.053 | 0.013 | 0.045[b] |
| mg kg$^{-1}$ BW | 14.8 | 17.1 | 11.3 | 11.4 | 33.0 | 2.46 | 0.084 | 0.014 | 0.044[c] |
| mmol kg $^{-0.75}$ BW | 0.31 | 0.36 | 0.24 | 0.25 | 0.71 | 0.05 | 0.053 | 0,013 | 0.045[d] |

Significant: ***(*P*<0.001); **(*P*<0.01); *(*P*<0.05); [a] Ŷ = 0.6470 (±0.06) *** + 0.0158 (±0.006) X *; [b] Ŷ = (0.1308±0.0151) *** + (0.0292±0.0140) X - (0.0039±0.0016) $X^2$ * + (0.00012±0.00005) $X^3$ *; [c] Ŷ = (14.8002±1.7056) *** + (3.3050±1.5872) X * - (0.4393±0.1824) $X^2$ * + (0.0134±0.0050) $X^3$ *; [d] Ŷ = (0.3060±0.02834) *** + (0.0729±0.0346) X * - (0.0096±0.0040) $X^2$ * + (0.0003±0.0001) $X^3$ *.

Table 4 shows the results of purine derivatives excretion from daily creatinine excretion obtained for each individual diet, such as averages presented in Table 3. The quadratic and cubic components were significant (P<0.05) for the total purine derivatives excretion, absorbed purines and microbial synthesis. However, for absorbed purines, microbial synthesis and efficiency the regression analysis showed that only the quadratic regression equations adjusted significantly (P<0.05) to the means. For excretion of purine derivatives a cubic equation was significantly adjusted, which showed maximum points at 5% and 18% RTPM replacing maize in the diet. It was estimated minimum microbial nitrogen synthesis and microbial efficiency in the rumen at the RTPM level around 9%.

Table 4. Urinary excretion of uric acid, allantoin, xanthine-hypoxanthine (H-X) and total purine derivatives, absorbed microbial purines, microbial nitrogen synthesis and microbial efficiency in lambs fed rain tree pod meal levels replacing maize

| Item | Replacing level (% DM) | | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 15 | 20 | 25 | SEM | L | Q | C |
| Urinary excretion (mmol kg$^{-1}$ BW$^{0.75}$ day$^{-1}$ ) | | | | | | | | | |
| Uric acid | 0.03 | 0.04 | 0.02 | 0.01 | 0.03 | 0.004 | 0.68 | 0.32 | 0.04[a] |
| Allantoin | 0.19 | 0.24 | 0.09 | 0.11 | 0.46 | 0.03 | 0.002[b] | 0.001 | 0.001 |
| X-H | 0.08 | 0.18 | 0.03 | 0.05 | 0.11 | 0.02 | 0.49 | 0.51 | 0.07 |
| Purine derivatives | 0.29 | 0.41 | 0.19 | 0.18 | 0.60 | 0.04 | 0.07 | 0.002 | 0.002[c] |
| Purine bases (mmol day$^{-1}$) | 11.8 | 16.1 | 7.4 | 6.4 | 19.1 | 1.91 | 0.08 | 0.004[d] | 0.001 |
| Microbial nitrogen (g day$^{-1}$) | 8.6 | 11.7 | 5.4 | 4.7 | 19.1 | 1.39 | 0.08 | 0.004[e] | 0.001 |
| Microbial efficiency (g CP kg$^{-1}$ TDN) | 93.6 | 139.6 | 59.1 | 57.8 | 214.5 | 15.89 | 0.06 | 0.004[f] | 0.002 |

Significant: *** (*P*<0.001); ** (*P*<0.01); * (*P*<0.05); [a] Ŷ = (0.028±0.0023) *** + (0.0080±0.0038) X * - (0.0011±0.0004) $X^2$ * + (0.000031±0.000011) $X^3$ *; [b] Ŷ= (0.2112±0.0318) *** - (0.0056±0.002) X ** ; [c] Ŷ = (0.2864±0.0519) *** + (0.1173±0.0342) X ** - (0.0140±0.0037) $X^2$ ** + (0.0004±0.0001) $X^3$ **; [d] Ŷ = (12.7906±3.1355) *** - (1.2597±0.5890) X * + (0.0685±0.0252) $X^2$ **; [e] Ŷ = (9.30±2.28) *** - (0.92±0.43) X * + (0.05±0.02) $X^2$ **; [f] Ŷ = (98.37±19.95) *** - (9.37±4.41) X * + (0.48±0.21) $X^2$ *.

## DISCUSSION

Possibly, RTPM levels above 58g $kg^{-1}$ diet dry matter affected the kidney function which resulted in the cubic variation of daily creatinine excretion by the animals in the present study. This can be explained by the fact that rain tree pods have secondary compounds of various classes, such as pithecolobin alkaloid, saponin, flavonoids, condensed tannins and some of these compounds have been implicated in altering renal activity (Shokunbi *et al*., 2008; Obasi *et al*., 2010; Ukoha *et al*., 2011; Karin and Azlan, 2012; Roshni *et al*. 2014). Thus, it can be inferred that the renal response is dependent of the dose of bioactive secondary metabolites from the rain tree (Ukoha *et al*., 2011).

According Chizotti *et al*. (2008), the creatinine excretion does not vary depending on the diet and can be excreted in urine in a manner constant relative to muscle mass in cattle. However, Pereira *et al*. (2013a) and Pereira (2015) found variations in daily creatinine excretion as a reflection of the changes in endogenous creatinine clearance in sheep fed diets containing maize replaced by peach palm meal levels and mesquite pod meal, respectively.

The increase in urine volume with RTPM levels can be explained by the presence of phytochemicals in the pod. It corroborates with Suresh *et al*. (2010), who suggested that the presence of flavonoids and steroidal compounds in the methanol extract of the bark of rain tree may be responsible, at least in part, for the diuretic activity. Likewise, Rhiouani *et al*. (1999) showed that the use of saponins altered the glomerular filtration rate in hypertensive rats, causing an increase in urinary flow.

Many factors interfere with urine volume. Without a doubt, water-deficient states will decrease the production of urine, due to less blood supply to the kidneys and more water reabsorption in the renal tubules. To reduce the enormous influence of urine dilution, the creatinine concentration is analysed with the substance that is to be measured since the total amount of creatinine formed in the body and excreted in the urine depends on body weight and amount of muscle mass. It has been recommended that, to correct weight discrepancies between animals, the daily creatinine excretion is corrected for body weight (Chen *et al*., 1992). Creatinine is freely filtered by the glomerulus and is found in the glomerular filtrate in the same concentration as in the blood. Another advantage of creatinine is that it is not absorbed and is secreted very little into the renal tubules, thus indicating the glomerular filtration rate (Pitts, 1974).

Even if there is variation of urine volume, daily creatinine excretion does not change, since the glomerular filtration remains constant. In this sense, it could indicate the use of a reference index from an animal species and/or breed for daily creatinine excretion per body weight as an indicator of urine volume, in case of impossibility to perform the total collection of 24 h-urine (Chen *et al*., 1992). However, in the present study the use of RTPM levels in the diets affected the daily excretion of creatinine, making it unfeasible to use single average value for creatinine daily excretion to estimate the urine volume (Pereira, 2015).

Pereira *et al*. (2013b) and Pereira (2015) reported that the change of the glomerular filtration rate can interfere with accuracy of the technique of purine derivatives excretion to estimate the microbial protein synthesis, thereby, requiring an assessment of daily creatinine excretion in different diets.

Therefore, it proceeded to use daily creatinine excretion obtained for each diet as averages presented in Table 3, to calculate the volume of urine produced and to estimate the purine derivatives excretion using spot urine sample collected at 4 h after morning feeding. The results showed that RTPM levels above 10% inhibited microbial protein synthesis in the rumen, possibly caused by the presence of bioactive substances that have antimicrobial activity. Moreover, RTPM levels above the 20% replacement of the maize affected renal physiology, overlapping the action of RTPM levels as an inhibitor of microbial protein synthesis in the rumen. According to Chen and Ørskov (2004), it is necessary to evaluate the effect of renal physiology on the excretion of purine derivatives, focusing on the identification of factors affecting the excretion of purine derivatives from plasma and the effect of glomerular filtration rate, secretion and reabsorption.

The supplementation with RTPM at 60g $kg^{-1}$ of total DM intake was beneficial for lactating dairy cows fed 40% roughage and 60% concentrate, resulting in improved purine derivative (allantoin) excretion and enhanced efficiency of rumen microbial CP synthesis (Anantasook *et al.*, 2014). Anantasook *et al.* (2015) related that RTPM contained condensed tannins and crude saponins at 88 and 141g $kg^{-1}$ of DM respectively, reducing the population of methanogens and protozoa in the rumen, increasing the population of *Fibrobacter succinogenes*, while *Ruminococcus flavefaciens* and *R. albus* were not affected. Ukoha *et al.* (2011) demonstrated the inhibitory effect of tannin from rain tree on several species of gram-positive bacteria. Obasi *et al.* (2010) reported an antimicrobial activity pod extract of rain tree on *Staphylococcus aureus* and *Bacillus subtilis*.

The means presented in this study for microbial synthesis efficiency were lower than that suggested as adequate by the NRC (Nutrient…, 2001) for cattle, which is 130g CP $kg^{-1}$ NDT, except for the levels of 10% and 25% RTPM. Therefore, there is evidence in this present study that RTPM caused enhanced microbial synthesis in the rumen shown by the estimated maximum point of purine derivatives excretion at 5% RTPM replacing maize. In contrast, possibly the 25% level of RTPM resulted in increased microbial protein synthesis as a reflection of high excretion of purine derivatives because of altered renal activity.

Secondary compounds (tannins and saponins) in RTPM did not inhibit dry matter intake but rather, had the potential to increase the nutrient digestion efficiency, probably due to the great effect on reducing protozoa and improving dietary nitrogen utilization (Anantasook *et al.*, 2014). It has been observed in the present study that even with increasing substitution of maize by RTPM, there was no reduction in intake of dry matter, crude protein and metabolizable energy. However, the concentration of total digestible nutrient had maximum point at the level of 9% replacement of maize by RTPM, which is consistent with the enhanced mean of microbial efficiency at 10% RTPM replacing maize.

In conclusion, the RTPM levels in the dry matter of the diet affect the daily creatinine excretion, compromising the accuracy of the purine derivative technique for estimating microbial synthesis. The supply above 58 g $kg^{-1}$ increases the excretion of total purine derivatives in the urine of lambs, as a consequence of a probable change in renal activity. RTPM added up to 29 g $kg^{-1}$ is beneficial for lambs, resulting in enhanced efficiency of rumen microbial crude protein synthesis.

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