Effect of propolis product on digestibility and ruminal parameters in buffaloes consuming a forage-based diet

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Abstract

This study evaluated the Propolis product (LLOS) effects on feed intake, dry matter (DM) and nutrients total digestibility, rumen characteristics and microbial efficiency in buffaloes fed a roughage based diet (70% Cynodon spp hay and 30% concentrate). Using a 4×4 latin square design, four crossbred buffalo (Murrah x Jafarabadi) steers (519.0±13.0 kg body weight - BW), were fed four treatments with three LLOS concentrations: Control (no LLOS), LLOS B3+ (0.272 mg/g flavonoid chrysin equivalents), LLOS C1 (0.092 mg/g flavonoid chrysin equivalents), and LLOS C1+ (0.184 mg/g flavonoid chrysin equivalents). Diet formulation was 60% total digestible nutrient (TDN) and 11% crude protein (CP). No difference was observed in DM intake among experimental diets (P>0.05). Steers fed LLOS C1 had greater (P<0.05) coefficients of digestibility for DM (67.7 vs 62.7%), organic matter (OM) (68.1 vs 63.7%), neutral detergent fibre (NDF) (61.7 vs 56.6%), total carbohydrates (TCHO) (67.3 vs 63.2%) and TDN (66.1 vs 62.8%) compared to animals fed the control diet. LLOS C1 provided greatest (P<0.05) short chain fatty acids (SCFA) production (141.89 vs 129.15 µM/mL) and butyric production (16.15 vs 14.05 µM/mL) compared to control. The lowest rumen pH (6.65; P<0.05) was observed in steers fed diets with LLOS C1. No difference (P>0.05) was observed for ammoniacal nitrogen (N-NH3), solid and liquid passage rate and microbial efficiency among treatments. In this study LLOS C1 improved forage diet efficiency in buffalo steers.

Introduction

Buffalo (Bubalus bubalis) feeding programs are primarily based on a high roughage diet. Valadares Filho and Pina (2006) suggested that animals fed with diet based on fodder increase rumen fermentation and the production rate of H2 and formate which are methane precursors. Animals fed high forage rations may release 13% of dietary energy as methane (Lana et al., 1998). Ionophore has been used as a promoter that increases feed efficiency and induces alteration in rumen fermentation patterns, which lead to reduce methane production. The use of propolis as an alternative to the ionophores for ruminant diets has being suggested by Oliveira et al. (2006) and Stradiotti Jr. et al. (2004). Lu et al. (2004) and Yang et al. (2007) suggested advantages of propolis anti-microbial properties specially against Gram-positive bacteria. European Union regulations currently restricts the use of ionophores in animal diets, however, propolis may be used to meet these milk and meat market requirements. However, until recently, published research in Brazil has not shown a standard concentration of propolis that can be used in an animal diet, nor the alcohol concentration to be used in the extraction of the active compounds and dosage of propolis to be used in animal feed. Thus, there are differences in the results from one experiment to another.

To standardize a product made from propolis, a dry propolis extract LLOS* (PI 0605768-3) was produced and added to buffaloes diets. Chromatography technic with high efficiency (HPLC) quantified phenolic compounds with anti-microbial activity (Prado, 2005) and with antioxidant activity (Cottica et al., 2011). Prado et al. (2010b) previous study tested propolis products in buffaloes diet, the most promising were LLOS C1 (propolis concentration C and alcoholic content 1) following by LLOS B3 (propolis concentration B and alcoholic content 3). Therefore, the objective of this study was to evaluate the effect of LLOS product with distinct concentrations of propolis; LLOS B3+, LLOS C1, LLOS C1+ in the ration of buffalo steers consuming a Cynodon spp. based ration upon nutrient use and digestibility kinetics.

Materials and methods

Four crossbreed buffalo steers (Murrah x Jafarabadi), 519.0±13.0 kg body weight (BW), fitted with rumen cannula, were used in 4×4 latin square design with four treatments and four periods of 29 days. Animals were kept in individual stalls with concrete floors, individual feeder and water supply. The animals were cared for according to the guidelines of the local animal committee of the Universidade Estadual de Maringá, Paraná State, Brazil.

Buffaloes were fed with 70% Cynodon spp hay and 30% concentrate diet with 60% total digestible nutrient (TDN) and 11% crude protein (CP) (Table 1). The distinctions between the experimental diets were the inclusion or not of additives (propolis products - LLOS): Control (no LLOS); LLOS C1; LLOS C1+; LLOS B3+. The LLOS differed in the concentration of propolis (B and C, between 5.0% and 30.0% (w/v) and water-alcohol solutions (1 and 3, between 60.0% and 93.8% (v/v) used for extraction the phenolic compounds. LLOS C1+ was twice LLOS C1 concentration of phenolic compounds; and LLOS B3+ was twice LLOS B3 concentration of phenolic compounds. The propolis extract prepared according to the methodology developed by Franco and Bueno (1999) were vacuum filtered and dealcoholized in a rotary evaporator (Buchi, model RT 210) to the limit of 15% ethanol. Then, they were dried by lyophilization. The propolis samples were obtained from the apiary located within a reserve of eucalyptus (Eucalyptus sp.) surrounded by native forest, with the presence of alecrim-do-campo (Baccharis dracunculifolia). More information about the preparation method, propolis and solvent concentrations are protected by a patent at the National
Institute of Industrial Property (INPI) under the number PI 0605768-3.

To create a standard dosage of propolis product LLOS (extract + excipient) in this experiment, we produced a dry propolis extract (C1 and B3). The two dry propolis extracts were evaluated by high performance liquid chromatography (Alliance HPLC-PDA system, Waters Co., Milford, MA, USA) and their quantifications and chemistry composition are shown in Table 2.

Total ration was provided twice a day in two equal portions, at 8.00 h and 16.00 h to allow for orts of 5% to 10% of total ration provided. Due the amount of extract supplied to the animals was small, than added an excipient (corn meal) with extract called up LLOS to add volume facilitate the animal feeding. The daily amount of flavonoid chrysin equivalents provided to the animals (through the LLOS products) were 0.272 mg/g to LLO B3+, 0.092 mg/g to LLOS C1 and 0.184 mg/g to LLOS C1+. An aliquot (five grams) of LLOS was weighed into a hygroscopic paper and added two intrarumen dosage daily immediately after feeding. To determine daily fecal dry matter, five grams of Chromium (III) oxide (Cr₂O₃) were provided at each feeding time.

Total experiment consisted of four periods, each experimental period last 29 days: 14 days to adapt animals, 8 days sampling and 7 days washout period. Steers were weighed at the beginning and the end of each sampling period. Orts samples were collected at 8.00 h and faeces (200 g) were collected directly from rectum at 8.00 h and 16.00 h from day 1st until day 5th of each collection period. Faeces and orts were frozen for subsequent analysis.

Rumen dilution rates were evaluated on day 6th of sampling; 30 g Co-EDTA were diluted in 500 mL distilled water and injected into rumen before the first feeding (Uden et al., 1980). Rumen fluid (100 mL) was collected from different rumen regions through rumen cannula. Measurement times were at 0.00, 2.00, 4.00, 6.00, 8.00, 10.00, 12.00, 14.00, 16.00 and 24.00 h after morning feeding. Rumen pH was measured immediately after sampling rumen fluid at 0.00, 2.00, 4.00, 6.00, and 8.00 h. A total of 50 mL of rumen fluid was collected and measured for Co concentration; 25 mL were acidified with 0.5 mL sulphuric acid 1:1 to measure N-NH₃ concentration; and other 25 mL of rumen fluid were acidified with 6.2 mL metaphosphoric acid (25%) to determine short chain fatty acid concentrations. Rumen fluid samples were labeled and storage in plastic bags at -20°C. To determine rumen microbial production urine samples were collected during spontaneous urination on day 6th of the collection period approximately four hours after feeding. Samples were filtered using filter paper. To prevent rumen purine derivate (PD) destruction due to uric acid precipitation, a 15 mL aliquot of urine was diluted in 135 mL of 0.036 N sulphuric acid (H₂SO₄) and samples were labeled and stored at 5°C for subsequent analysis. To determine gastrointestinal solid kinetic and rumen fluid volume, the rumen contents were evacuated on 7th and 8th days of the collection period. Rumen evacuation started 4.00 h after first feeding and an hour before the second feeding (Huhtanen et al., 2007). Total rumen content were manually pressed, solid and liquid parts were separated and measured. Proportional (w/w) solid and liquid samples (2.0 kg) were collected to determined rumen DM percentage. Samples were dried at 55°C and mixed for analysis. Rumen evacuation, pressing rumen samples and the return of the remaining contents were approximately 20 min.

Feed, orts and faeces for each animal/period/diet were dried at 55°C for 72 h and individually milled (1 mm). Chromium concentration in the faeces was determined by atomic absorption spectrometry after nitro-perchloric digestion (Kimura and Miller, 1957). Rumen fluid samples were filtered to determine ammonia concentration (Preston, 1995). To determine SCFA, samples were centrifuged at 10,000 xG (4°C) for 40 min. Analysis was then conducted according to Palmquist and Conrad (1971) using liquid-gas chromatography (Hewlett Packard 5890 Series II GC) associated with an integrator (Hewlett Packard 6890 Series Injecter). Chromatography sample reading were processed with 100 L 2-methylbutyric acid gradually added into each tube with 800 L of sample. Standard mixes of 76 short chain fatty acid with known concentration were used for calibration and peak identification.

To determined Co concentration of rumen fluid samples were thawed, centrifuged at 3700 xG for 30 min, and measured using atomic absorption spectrometry (Uden et al., 1980). An uni-compartmental exponential model by Hungate (1966) and Colucci (1984), adjusted liquid passage rate and cobalt concentration curves as follows, was used: YCo = A x e( -kt ); Where YCo = concentration in time indicator t; A = Co concentration equilibrium; t = passage rate or Co dilution; and t = sample time. Liquid phase dynamics were calculated according to Colucci et al. (1990): Rumen fluid reten-

### Table 1. Composition and chemical analysis of the diet.

|                | DM | OM | CP | EE | NDF | ADF | TCHO | NFC | % Diet |
|----------------|----|----|----|----|-----|-----|------|-----|--------|
| Cynodon hay, % DM | 91.94 | 93.0 | 9.6 | 1.0 | 72.2 | 42.8 | 81.8 | 8.02 | 70.0 |
| Soybean meal, % DM | 90.8 | 93.2 | 49.9 | 2.0 | 14.9 | 8.5 | 8.5 | 41.4 | 4.4 |
| Corn milled, % DM | 90.4 | 98.9 | 8.9 | 3.5 | 9.9 | 3.7 | 86.6 | 77.7 | 25.1 |
| Mineral salt, % DM | 90.6 | - | - | - | - | - | - | 0.6 |
| Total diet (70:30) a | 91.4 | 94.1 | 11.1 | 1.6 | 53.3 | 31.1 | 80.8 | 26.3 | 100.0 |

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; TCHO, total carbohydrates; NFC, non-fibre carbohydrates. aRatio total forage:concentrate.

### Table 2. Chemical composition of propolis extract.

|                | mg/g dry extract (hydrophilized) |
|----------------|----------------------------------|
|                | B3                               | C1                               |
| Chlorogenic acid | 2.12                             | nd                               |
| Caffeic acid    | 18.90                            | 6.24                             |
| P-coumaric acid | 39.75                            | 13.48                            |
| CAPE a           | 7.57                             | 4.96                             |
| Artepillin C b   | 29.81                            | 31.06                            |
| Apigenin         | 5.75                             | 5.19                             |
| Phloemebrine     | 7.27                             | 5.01                             |
| Galangin         | 3.43                             | 4.90                             |
| Chrysin          | 6.58                             | 9.70                             |
| Acacetin         | 9.87                             | 6.28                             |
| Flavonoid chrysin equivalents | 0.136 | 0.092 |

aCAPE, caffeic acid phenethyl ester; bArtepillin C, 3,5-diprenyl-4-hydroxycinnamic acid; nd, not detected.
tion time (h) \((\text{TeR}) = 1/\text{fluids passage rate (h)}\) (kCo); rumen liquid volume (L) \((\text{RLV}) = \text{Co amount provided (mg/A)}; \text{Rumen fluid rate (RFR) (L/h)} \) (TxF) = kCo x RLV and recyle rate (TRec) of rumen liquid phase were calculated according to Maeng and Baldwin (1976): In this equation, TRec (number of times/day) = 24h/TeR.

Gastrointestinal solid kinetic parameter was estimated using Robinson et al. (1987) calculation, as: ingestion rate/day [\( \text{ki} = \) (nutrient ingestion/rumen nutrient pool)*100]; passage rate/day [\( \text{kp} = \) (fecal nutrient excretion/rumen nutrient pool)*100]; digestion rate/day [\( \text{kd} = \text{ki} - \text{kp} \)]; Disappearance rate of solids/day = \([\text{kd} = \text{ki} + \text{kp}]\); and Retention time of solids (hours) = \([\text{Rt} = 100/\text{Ki}]\).

To obtain PD concentration from urine allantoin analysis were performed using the Chen and Gomes (1992) methodology. Creatine and uric acid were determined at Diagnostic Laboratorial Center (CEDLAB, Maringá, Paraná State, Brazil). Creatinine concentration was estimated from urine volume (L) divided by daily creatinine excretion mmol/kg BW\(^{0.75}\) per creatinine concentration mmol/L. To determine daily creatinine excretion (mmol/kg BW\(^{0.75}\)) 0.44 mmol/kg BW\(^{0.75}\) value was adopted according to Chen et al. (1996). Microbial nitrogen production were calculated from amounts of purine absorption (X mmol/day) which was estimated from PD urinary excretion (Y mmol/day) calculated by equation described by Dipu et al., (2006) for buffaloes: \(Y = 0.74X + (0.117 \text{BW}^{0.75})\). The 0.74 value represents purine that were recupered from PD urine absorption and 0.117 mmol/kg BW\(^{0.75}\)day was a constant value that represents PD from endogenous liquid contribution.

Rumen microbial nitrogen compound synthesis (Y g N/day) was calculated from the purine absorption ratio (X mmol/day) x 70/((0.116 x 0.83 x 1000)). Considering that 70 represents purine N content (mg N/mmol); 0.83 microbial purine digestibility and 0.116 represents total rumen microorganism N:purine:N ratio.

Microbial CP (CPmic) estimate was obtained by multiplying microbial N synthesis by 6.25, while the efficiency of microbial protein synthesis was determined as: ECPmic (g/100 g) = CPmic (g)/CTN (100 g), which TNDC = total nutrient digestive consumption.

DM, ash, CP and ethers extract (EE) rates were determined by AOAC (1990) and OM was obtained by subtracting ash from DM. NDF and ADF analysis were performed as described by Van Soest et al. (1991). NDF residues and neutral detergent insoluble nitrogen (NDIN) were determined following procedure by Licitra et al. (1996). TCHO were quantified by equation: 100 - ( %CP + %EE + %Ash). Non fibre carbohydrates (NFC) levels were obtained by subtracted TCHO and NDFDP rates, NFC is composed by cell wall without CP (Sniffen et al., 1992). SCFA, pH and N-NH\(_3\) values were obtained by a subdivision of sample/time experimental portions ratio. Regression analysis was used to determine SCFA, pH and N-NH\(_3\), considering time after morning feeding (0, 2, 4, 6 and 8 h).

**Table 3. Dry matter and diet nutrients intake.**

|                | Control | LLOS C1 | LLOOS C1+ | LLOOS B3+ | P     | SE    | VC  |
|----------------|---------|---------|-----------|-----------|-------|-------|-----|
| DM\(^{g}\) | kg/day  | 8.44    | 8.04      | 8.68      | 8.44  | 0.32  | 0.13| 5.85 |
| %TBW         |         | 1.57    | 1.51      | 1.62      | 1.52  | 0.21  | 0.02| 4.79 |
| g/MW\(^{g}\) |         | 75.76   | 73.75     | 77.88     | 73.04 | 0.33  | 0.13| 5.07 |
| OM\(^{g}\)   | kg/day  | 7.95    | 7.56      | 8.16      | 7.69  | 0.31  | 0.12| 5.80 |
| %TBW         |         | 7.56    | 7.12      | 7.32      | 7.67  | 0.21  | 0.09| 4.81 |
| NDF\(^{g}\)  | kg/day  | 4.73    | 4.47      | 4.89      | 4.59  | 0.32  | 0.08| 6.61 |
| %TBW         |         | 0.88    | 0.87      | 0.91      | 0.85  | 0.48  | 0.02| 6.02 |
| CP            | kg/day  | 0.95    | 0.92      | 0.92      | 0.92  | 0.31  | 0.02| 5.53 |
| EE            | kg/day  | 0.15    | 0.14      | 0.15      | 0.14  | 0.27  | 0.004| 4.72 |
| ADF\(^{g}\)   | kg/day  | 2.52    | 2.38      | 2.62      | 2.45  | 0.27  | 0.05| 6.71 |
| TCHO          | kg/day  | 6.81    | 6.46      | 6.98      | 6.58  | 0.32  | 0.32| 5.90 |
| NFC           | kg/day  | 2.38    | 2.28      | 2.41      | 2.29  | 0.39  | 0.03| 5.22 |
| TDN           | kg/day  | 4.08    | 4.30      | 4.52      | 4.18  | 0.34  | 0.08| 7.90 |

VC, variable coefficient; DM, dry matter; OM, organic matter; %TBW, total body weight; NDF, neutral detergent fibre; CP, crude protein; EE, ethers extract; ADF, acid detergent fibre; TCHO, total carbohydrates; NFC, non-fibre carbohydrates; TDN, total digestive nutrients. \(^{a,b}\)Means with different superscript letter in the same row are significantly different (P<0.05).
forage:concentrate.

Low feed intake was observed and can be attribute to diet composition of 70% Cynodon ssp hay, 72% NDF, 43% ADF and 10 cm particle size. However, CP and TDN animal intake were higher than maintenance requirements, 0.556 kg CP/day and 3.65 kg TDN/day for 500 kg BW buffaloes (Kearl, 2003). This was evidenced by the weight gain of 230 g/day during 112 days experimental period.

LOLS C1 increased (P<0.05) DM, OM, NDF, TCHO and TDN digestibility coefficient (Table 4). No differences were observed for CP, EE, ADF and NFC digestibility coefficient among diets (P>0.05). Prado et al., (2010b) also observed DM higher digestibility coefficient with LOLS C1 vs. control diet (62.8 vs 59.4%) respectively. Additionally, Prado et al., (2010b) also observed improved OM, NDF, TCHO and TDN digestibility coefficient values in buffaloes consuming an 80:20% forage:concentrate ration supplemented with LOLS C1.

LOLS C1, LOLS C1+ and LOLS B3+ all increased NDF digestibility coefficient (P<0.05) compared to control diet (Table 4), which indicates that fibrolytic microorganisms are tolerable to difference dosage and concentrations of propolis products. Prado et al., (2010b) observed a greater NDF digestibility coefficient in LOLS C1 compared with monensin and control diet. Prado et al., (2010a) in a previous in vitro study, evaluated microbial strains tolerable to propolis products suggesting that LOLS C1 selected for rumen bacteria capable of degrading cellulose, cellulose, aminoxylose, fructose, lactose and glucose.

LOLS C1 compared to control diet had a greater TDN (P<0.05) (66.1% vs 62.8%) (Table 4), which are similar to the observed (65.8% vs 62.3%) by Prado et al. (2010b). Among the cellulosytic strains (Fibrobacter succinogenes S85, Ruminococcus flavefaciens FD-1, Ruminococcus albus 7, Ruminococcus albus 20 and Butyrivibrio fibrisolvens DSMZ 3071) tested in vitro by Aguilar et al. (2012), the Fibrobacter succinogenes S85 and Ruminococcus flavefaciens FD-1 showed high sensitivity to the LOLS C1. These results may indicate that ruminal cellulosalactic activity would be reduced by higher doses of phenolic compounds which may explain the high TDN value for LOLS C1.

Diets and time interfered on rumen pH (P<0.05). LOLS C1 had significance different compared other treatments (P<0.05), but no difference was observed among control diet, LOLS C1- and LOLS B3- (Table 5). The pH average was 6.71 which are considered by Van Soest (1994) an optimal value for cellulosalactic bacteria growth. LOLS C1 had the lowest rumen pH of 6.44 (P<0.05) at 8 h (Figure 1), however, is considered above the critical pH value of 6.2 (Van Soest, 1994). It is likely that the relatively stable and elevated pH values are a result of the slowly fermentable roughage diet. Furthermore, buffaloes present high salivary secretion resulting better ruminal buffer (Sivkova et al., 1997).

Ammonial nitrogen (N-NH3) in the rumen fluid showed cubic effect (P<0.05) over time. However, no differences were observed in N-NH3 concentration among diets, nor differences of diet and time (P>0.05). The greatest N-NH3 production was 21.10 mg/100 mL at 2h12 min and the lowest production was 10.07 mg/100 mL at 6h37 min, measured after morning feed event (Figure 1). Minimal acceptable N-NH3 concentrations suggested by Hoover (1986) range from 3.3 to 8.0 mg/100 mL, and maximum rumen fermentative activity suggested by Mehrez et al. (1977) interval were indicated to be between 19 to 23 mg N-NH3/100 mL. Considering that buffaloes high salivary production may contribute to urea recycling and keep the ruminal ammonia levels, which help on ruminal fermentation (Tewatia and Bhatai, 1998; Trufchev et al., 1997).

LOLS C1 had greater concentrations of total SCFA production (P<0.05) compared to control diet, with the exception of acetate:propionate ratio production (Table 5). The higher fibre carbohydrates digestibility probably resulted in high SCFA production with LOLS C1. Propionate concentrations were greater (P<0.05) with the addition of LOLS C1 compared with LOLS C1+ (LOLS C1+ had twice the amount of LOLS C1 added). Extra addition propolis (LOLS C1+) may have reduced the population of bacteria that produce propionate. Elevated SCFA productions were observed with propolis products added in buffaloes ration compared with literature values, and similar

| Table 4. Digestibility coefficient and total digestible nutrients in diets with propolis product. |
|-------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Diet              | Control          | LOLS C1          | LOLS C1+         | LOLS B3+         |     P   |     SE  |     VC  |
|-------------------|------------------|------------------|------------------|------------------|--------|--------|--------|
| DM, %             | 62.7±            | 67.2±            | 66.2±            | 66.1±            | 0.030  | 0.80   | 2.45   |
| OM, %             | 63.7±            | 68.1±            | 67.1±            | 66.9±            | 0.033  | 0.80   | 2.39   |
| CP, %             | 64.2             | 68.1             | 68.8             | 67.1             | 0.253  | 1.50   | 4.49   |
| NDF, %            | 56.6±            | 61.7±            | 60.0±            | 60.7±            | 0.051  | 0.61   | 2.05   |
| EE, %             | 71.4             | 77.9             | 76.0             | 75.6             | 0.429  | 2.62   | 6.96   |
| NFC, %            | 76.5             | 76.3             | 78.2             | 77.4             | 0.941  | 2.59   | 6.51   |
| TDN, %            | 62.8±            | 66.1±            | 65.6±            | 65.5±            | 0.038  | 0.83   | 1.95   |

VC, variable coefficient; DM, dry matter; OM, organic matter; %TBW, total body weight; ND, neutral detergent fibre; CP, crude protein; EE, ethers extract; ADF, acid detergent fibre; NFC, non-fibre carbohydrates; TDN, total digestive nutrients. a,bMeans with different superscript letter in the same row are significantly different (P<0.05).

| Table 5. Rumen pH, ammonical nitrogen and short chain fat acids (µM/mL) production average values, ratio acetate:propionate. |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| Diet              | Control          | LOLS C1          | LOLS C1+         | LOLS B3+         |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| pH                | 6.76             | 6.65             | 6.73             | 6.73             |
| N-NH3             | 16.29            | 14.94            | 15.25            | 15.12            |
| SCFA              | 129.15           | 141.88           | 130.07           | 133.49           |
| Acetate           | 69.23            | 97.73            | 91.93            | 93.39            |
| Propionic         | 49.98            | 55.14            | 52.89            | 56.54            |
| Butyric           | 11.08            | 16.15            | 13.60            | 14.57            |
| Isovaleric        | 21.49            | 21.09            | 20.10            | 20.16            |
| Valeric           | 1.18             | 1.50             | 2.06             | 2.06             |
| Isobutyric        | 1.18             | 1.50             | 1.33             | 1.51             |
| Valerolic         | 1.18             | 1.50             | 1.33             | 1.51             |
| Acetate:propionate| 1.56             | 1.98             | 2.12             | 2.18             |

VC, variable coefficient; N-NH, ammonial nitrogen; SCFA, short chain fat acids. a,bMeans with different superscript letter in the same row are significantly different (P<0.05).
concentration of NDF. Prado et al., (2010b) observed 73.01 µM/mL, 52.58 µM/mL, 12.98 µM/mL and 7.94 µM/mL for total SCFA, acetate, propionate and butyrate respectively, in diets with 55% of NDF. Souza et al., (2000) obtained 75.01 µM/mL, 55.05 µM/mL, 13.26 µM/mL and 6.27 µM/mL for total SCFA, acetate, propionate and butyrate respectively, in buffaloes fed diets with 54% of NDF. According France and Dijkstra (2005) the total volatile fat acids is normally between 70 and 130 mM and the fermentation is determined by the composition of the microbial population, which is turn is largely determined by the basal diet. High-fibre forage diets encourage the growth of acetate producing bacterial species. The high-fibre diet associated with LLOS C1 could have contributed to fibrolytic microorganism growth which increased the total SCFA concentration.

Propolis product LLOS C1 increased butyrate (P<0.05) concentration compared with LLOS C1+, LLOS B3+ and control diet. Van Nevel and Demeyer (1988) suggested that high propionate concentrations leads to low concentrations of butyrate, however, in this experiment, propionate and butyrate concomitantly increased.

Among diets significant difference (P<0.05) were observed in rumen of branched chain fatty acids (BCFA) production (table 5). BCFA are generated from deamination of branched chain amino acids (valine, leucine and isoleucine) and constitute important factors for growth of many fibrolytic microorganisms.

Among LLOS products, LLOS C1 showed highest production of isovalerate which was similar to control diet. Isovalerate synthesis is a derivative process from leucine oxidative deamination which is an important source of carbon supply to cellulolytic bacteria development (Val Neto, 2009). The production of isobutyrate was lower to LLOS C1+ (P<0.05) compared other diets, but no difference was observed among other diets. Therefore, the lowest concentration of BCFA in LLOS C1+ and LLOS B3+ may reflect the ruminal metabolism of protein. Strains Prevotella albensis and P. ruminicola, both proteolytic, showed sensitivity to the propolis extracts B1, C1 e C3 only at highest concentration tested; however, strains of Peptostreptococcus sp. and C. aminophilum, both hyper-ammonia producing bacteria, showed high sensitivity to LLOS at different concentrations, except for the lower concentration (Aguirar, et al. 2012). The results of this study are agreement with Aguiar et al. (2012) because the active principles of propolis reduce protein fermentation in higher concentrations of products.

Addition of propolis products in buffaloes
diet shown no differences (P>0.05) on rumen solid and liquid dynamics (Table 6). Solid and liquid passage rate are influenced by forage:concentrate ratio and daily intake (Poncet, 1991). Valadaires Filho and Pina (2006) showed that solid and liquid passage rates increased proportional to forage ratio and feed intake.

Liquid passage rate was 13.11%/hour, higher than observed by Maeda et al. (2007) 12.2%/h in buffaloes fed sugar cane silage with 60:40 forage:concentrate ratio with 1.48% BW intake. Souza et al. (2009) observed 9.6%/h liquid passage rate in buffaloes fed 85% sugar cane forage diet with 0.91% BW intake. Rumen liquid recycle rate was 3.15 times a day, Nogueira Filho et al. (2004) observed 3.85 times a day in buffaloes fed 10.6 kg DM intake with 65% Cydonon dutchyon hay and 35% concentrate. Low DM intake (8.4 kg) resulted in lower recycle rates. Liquid passage rate was 10.50 L/h, which was greater than observed by Prado et al. (2010b), 8.0 L/h in buffaloes fed with 70% corn silage, 10% Tifton hay and 20% concentrate. This may have been influenced by the lack of moisture in the diet, thereby increasing salivary secretion and increased liquid passage rate. Rumen volume obtained by cobalt dilution methodology was 12 L higher than rumen evacuation through fistula (80 vs. 70L) obtained 15.5% BW vs 13.1% BW (Table 6). Franzolin et al. (2002) observed rumen volume of 13.6% BW in diets with 70% of NDF; Prado et al. (2010b) observed 13.8% BW in diets with 55% of NDF; therefore, rumen evacuation via fistula is considering the most accurate methodology of volume reported in buffaloes. Rumen dry matter was 15.3% BW which is the limit interval value (10% to 15%) cited by Coelho da Silva and Leão (1979), values fluctuates depending on sampling time, water ingested and diet composition. Gastrointestinal solid passage rate in buffaloes was 1.13%/h (Table 6); this would be considered low value based on ARC (1984) 2.0%/h, maintenance bovine recommendations. Terramoccia et al. (2000) observed 2.42%/h in buffaloes fed 75.25% forage:concentrate diet. Low value solid passage rate was associated with forage quality and feed intake.

Solid digestion rate was 2.14%/h (Table 6), which is lower than observed by Nocek and Grant (1987) with 3.0%/h in animals fed Ductylis glomerata L. forage. The high percentage of DM in this trial could have influenced the lower rate of digestion solid. Huhtanen et al. (2006) comments that rumen evacuation technique can influence at variation in the results at the ruminal digestion rate because ignores omosal cell wall pools, which has some influence on estimated kinetic parameters. Gastrointestinal solid retention time was 31.10 h (Table 6), less than time observed for Martins et al. (2006) with 38.56 h in bovines fed 68% Tifton 85 hay.

Experimental diets had no (P>0.05) alteration on microbial protein synthesis (g/day) and microbial protein synthesis efficiency (g/100g TDN), with 9.85 g/100g TDN (Table 7). These values are lower than suggested by NRC (1996) bovines 12.8 g/100g of TDN with 40% forage diet, however, roughage:concentrate ratio (70:30) are favorable to optimal pH, better passage rate and microbial colonization condition (Sniffen and Robinson, 1987). Average value for microbial efficiency protein synthesis were observed to be higher than suggested by Dípu et al. (2006) with 2.91 g/100g of TDN in 297 kg BW, 95% feed intake (4.75 kg of DM/day, 1.60% BW) buffaloes fed 40% wheat straw and 60% concentrate. Allantoin and uric acid excretion showed no alterations (P>0.05) among diets (Table 7) the proportion was 99.12% and 0.88%, respectively. Dípu et al. (2006) observed 91.46% and 8.54%; Chen et al. (1996) showed 90.64% and 9.36% and Cutrignelli et al. (2007) with 84.73% and 11.57% allantoin and uric acid proportions, respectively. Chen and Gomes (1992) suggested different concentrations of allantoin among animals are usually constant, however, an excretion chance was observed.

### Conclusions

Among propolis product, LLOS C1 appears to improve nutritional value in buffaloes by altering fermentation kinetics. It should also be noted that greater dosages of the propolis product may lose this effect.

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### Table 7. Microbial synthesis and microbial efficiency synthesis (g MP/100g of TDN) in different diets.

| Diet       | Control | LLOS C1 | LLOS C1+ | LLOS B3+ | Average | P    | SE  | VC  |
|------------|---------|---------|----------|----------|---------|------|-----|-----|
| UV(L)      | 23.77   | 20.04   | 21.46    | 28.13    | 24.11   | 0.302| 2.31| 19.17|
| Purine derived in urine |         |         |          |          |         |      |     |     |
| ALA        | 76.54   | 77.61   | 85.74    | 80.82    | 80.18   | 0.753| 6.47| 16.13|
| UA         | 0.65    | 0.77    | 0.79     | 0.72     | 0.73   | 0.964| 0.22| 60.48|
| TP         | 77.18   | 78.38   | 86.53    | 81.55    | 80.91   | 0.757| 6.58| 16.27|
| ALA, %     | 99.18   | 99.11   | 99.04    | 99.17    | 99.12   | 0.968| 0.22| 45.46|
| UA, %      | 0.82    | 0.89    | 0.86     | 0.83     | 0.88   | 0.968| 0.22| 51.64|
| MPA        | 86.69   | 88.42   | 90.32    | 92.49    | 91.73   | 0.762| 8.94| 19.48|
| MNC        | 63.02   | 62.84   | 72.21    | 72.74    | 66.69   | 0.762| 5.50| 19.48|
| MPS        | 383.88  | 401.75  | 451.28   | 420.23   | 416.79  | 0.762| 40.61| 19.48|
| MES        | 9.84    | 9.36    | 9.95     | 10.26    | 9.85   | 0.951| 1.15| 23.46|

VC, variable coefficient; UV, urinary volume; ALA, allantoin (mmoL/day); UA, uric acid (mmoL/day); TP, total purines (mmoL/day); MPA, microbial purine absorbed (mmoL/day); MNC, microbial nitrogen compounds (g/day); MPS, microbial protein synthesis (g/day); MES, microbial efficiency synthesis (g MP/100 g of TDN); MP, microbial protein.
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