# Impact of propolis as a natural antibiotic on diet digestibility, purine derivatives, methane emission and blood constitutes of late pregnant ewes

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# Impact of propolis as a natural antibiotic on diet digestibility, purine derivatives, methane emission and blood constitutes of late pregnant ewes

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**Abstract.** Antibiotic are widely used in ruminant nutrition as feed additives to improve growth energy, protein utilization and decrease methane ($CH_4$) emissions. However, there is a controversy about the use of such additives because of the risk of transferring residues into meat and milk and development of resistant strains of bacteria. These concerns have promoted the search for alternative natural feed additives, such as propolis. Eighteen ewes were individually allotted to indoor pens to study the impact of propolis as a natural antibiotic on diet digestibility, purine derivatives, $CH_4$ emissions and blood constitutes of late pregnant Santa Inês ewes. The study lasted for 43 day and ewes were assigned to a randomized complete block design with 9 blocks and 2 dietary treatments as follows: Control (CTL) without propolis administration and propolis (BRP) received 3 g red propolis extract /ewe/day. Intake of N and body N retention increased ($p < 0.05$) for BRP supplementation than CTL expressed as g / animal / day. The administration of BRP increased ($P < 0.05$) urinary allantoin compared to the CTL. BRP supplementation increased ($P < 0.05$) OM and CP digestibility compared to the CTL. Additionally BRP decreased ($P < 0.05$) $CH_4$ emission expressed as L/day or L/kg for (OMI and DOM) when compared to CTL group. Propolis increased ($P < 0.05$) all hematological parameters moreover the same pattern was observed for the biochemical parameters too. Propolis supplementation resulted in decrease ($P < 0.05$) cortisol, $T_3$ and $T_4$ compared to CTL. Propolis represents a promising natural antibiotic with profit biological and environmental effects.

**Keywords.** Antibiotic – Digestibility – Propolis – Methane – Purine derivatives.

***Impact du propolis, un antibiotique naturel sur la digestibilité de la ration, les dérivés des purines, les émissions de méthane et les constituants du sang chez les brebis enfin de gestation***

***Résumé.*** *Les antibiotiques sont largement utilisés comme additifs alimentaires dans la nutrition des ruminants pour améliorer l'énergie de croissance, l'utilisation des protéines et pour réduire les émissions de méthane ($CH_4$). Cependant, l'utilisation de tels additifs pourrait être à l'origine du transfert de résidus dans la viande et le lait et du développement de souches bactériennes résistantes. Ces préoccupations ont favorisé la recherche sur les additifs naturels dans l'alimentation animale, tels que la propolis. Dix-huit brebis ont été individuellement logées dans des enclos pour étudier l'impact de la propolis en tant qu'antibiotique naturel sur la digestibilité de la ration, les dérivés des purines, les émissions de $CH_4$ et les constituants du sang chez les brebis de race Santa Inês en fin de gestation. L'étude a duré 43 jours et les brebis ont été affectées à deux groupes: le groupe témoin (CTL n=9) et celui qui recevait 3 g d'extrait de propolis rouge/ brebis / jour (BRP n=9). L'apport en azote et la rétention d'azote dans le corps ont augmenté ($p <0,05$) pour la supplémentation en BRP par rapport aux CTL exprimés en g par animal et par jour. L'administration de BRP a augmenté ($P < 0,05$) l'allantoïne urinaire par rapport à celui du CTL. La supplémentation en BRP a augmenté ($P < 0,05$) la digestibilité de la matière organique et celle des matières azotées de la ration par comparaison au régime témoin (CTL). De plus, les BRP ont diminué ($P <0,05$) les émissions de $CH_4$ exprimées en L / jour ou en L / kg pour (Quantité Ingérée de MO (MOI) et quantité de MO ingérée digestible (MOD)) par rapport au groupe CTL. La propolis a augmenté ($p < 0,05$) tous les paramètres hématologiques et le même schéma a été aussi observé pour les paramètres biochimiques. La supplémentation en propolis a entraîné une diminution ($P <0,05$) du cortisol, des $T_3$ et $T_4$ par rapport aux CTL. La propolis est un antibiotique naturel prometteur ayant des effets biologiques et environnementaux bénéfiques.*

***Mots-clés.*** *Antibiotique – Digestibilité – Propolis – Méthane – Dérivés de la purine.*

# I – Introduction

Antibiotic growth promoters are widely used in ruminant nutrition as feed additives to improve energy and protein utilization and decrease methane ($CH_4$) emissions from ruminants (Wischer *et al*., 2013). However, there is a debate about the use of antibiotics in animal diets because of the risk of transferring residues into meat and milk and promoting the development of resistant strains of bacteria. These concerns have promoted the search for alternative natural feed additives, such as propolis (Morsy *et al*., 2013; Morsy 2015). Propolis is known to be active against (gram positive) bacteria, viruses, fungi, oxidants, inflammation, tumors, and parasites, and it may act as an immunomodulator (Alencar *et al*., 2007; de Aguiar *et al*., 2013; Morsy *et al*., 2015). There are many studies that confirmed the suitability of propolis as an effective feed additive for ruminants. Zawadzki *et al*. (2011) concluded that propolis supplementation, improved feed efficiency and body weight gain in feedlot-finished bulls. Ítavo *et al*. (2011) reported similar findings in growth performance of feedlot lambs. Brazilian red propolis was beneficial for ewe's health during the flushing period (Morsy*et al*., 2013). *In vitro*, propolis was effective in decreasing the formation of ammonia in the rumen (Oeztuerk *et al*., 2010) and inhibited hyperammonia-producing species of bacteria (de Aguiar *et al*., 2013). The most characteristic of propolis compared to the dietary antibiotic that propolis can decrease the $CH_4$ emission while improve the ruminal fermentation and nutrient degradability (Morsy *et al*., 2015). However, little information on the effect of propolis on the microbial protein synthesis is available. Thus, the hypotheses of the present study were that propolis as a natural antibiotic can improve the feed digestibility and enhance the microbial protein synthesis, while decreasing $CH_4$ emission during the critical period of late pregnant ewes. The objective of the present study was to investigate the impact of propolis on diet digestibility, purine derivatives, $CH_4$ emission and blood constituents of late pregnant ewes.

# II – Materials and methods

## 1. Animals

This study was conducted in Centre for Nuclear Energy in Agriculture (CENA, USP) Piracicaba, Brazil.All animals were always treated in accordance to the Internal Commission for Environmental Ethics in Experimentation with Animals of CENA/USP (approval no. CIEEA/CENA 001/2011). Eighteen late pregnant Santa Inês ewes 52.5±2.50 kg body weight were individually allotted to indoor pens. The study lasted for 43 d and consisted of 14 d for adaptation and 29 d for data collection. Ewes were assigned to a randomized complete block design with 9 blocks and 2 dietary treatments. Control (CTL) without propolis administration and propolis (BRP) that received 3 g red propolis extract /ewe/day in the morning before access to diet for 21 days. Ewes were fed twice a day total mixed ration and formulated to meet NRC (2007). Total mixed ration is composed of (on DM basis) 50% tifton hay, 32.7% ground corn, 15.0% soybean meal, 1.0% limestone, and 1.3% mineral premix. The chemical composition of this ration includes (on DM basis); 92.4% DM, 13.1% CP, 2.0% EE, 4.3% ash, 71.8% NDF, and 34.3% ADF). Quantity of feed offered to animals was calculated according to previous daily DMI and adjustments were made when needed so that refused feed did not exceed 10% of the daily intake. Individual feed refusals were daily weighed to determine DMI.

## 2. Sampling and analysis

Feeds were sampled weekly throughout the trial and frozen at -20°C for further analyses. On the 7-day collection periods, the sheep were kept in metabolic cages, where complete individual daily collection of feed refusals, faeces and urine was performed to measure nutrient digestibility and N balance. Urine samples were analysed for N and for purine derivatives (i.e. allantoin, uric acid and creatinine) according to International Atomic Energy Agency (1997) by high performance liquid

chromatography (HPLC). The amount of microbial purines absorbed from the small intestine (PDa) was calculated according to Chen and Gomes (1992). At the end of the digestibility trial, animals were individually kept for two consecutive days in respiratory chambers for $CH_4$ determination as described by Abdalla *et al*. (2012). The $CH_4$ concentration was then determined on a gas chromatograph (GC) (Model 2014, Shimadzu, Tokyo, Japan) equipped with a Shincarbon ST 100/120 micropacked column (1.5875 mm OD,

1.0 mm ID, 1 m length; Ref. no 19809; Restek, Bellefonte, PA, USA). Blood samples (5 mL) were collected every week throughout experimental period before morning feeding. Samples were collected from the jugular vein using evacuated K2 EDTA tubes (Becton Dickinson & Co., Franklin Lakes, NJ) for hematological analyses and evacuated tubes without anticoagulant agents (Becton Dickinson & Co.) for biochemical analyses and hormones.

Blood serum concentrations of biochemical were determined by colorimetric kits using a commercial Labtest (Diagnóstica S.A®. Lagoa Santa, MG, Brazil). While hormones were analysed, by using radioimmunoassay (RIA) a commercial Siemens kit (Siemens Medical Solution Diagnostic, USA) using automatic Gama counter model (Wizard 2, Perkin Elmer, Dowens Grove, IL.USA). Data were analysed by analysis of variance using PROC MIXED procedure of SAS (2002).

## III – Results and discussion

The effect of BRP supplementation on apparent diet digestibility and $CH_4$ emission are presented in (Table 1). Brazilian red propolis supplementation increased ($P < 0.05$) OM and CP digestibility compared with the control. Additionally BRP decreased ($P < 0.05$) $CH_4$ emission expressed as L/day or L/kg for (OMI and DOM) when compared to the control group. Such finding is in line with that reported by Morsy *et al*. (2015), who demonstrated that BRP extracts promoted the ruminal degradation of nutrients, as can be seen from the increase in the truly degraded OM and decreasing $CH_4$ emission by increasing short chain fatty acids and the decrease in the protozoal count *in vitro.* Propolis can not only reduce $CH_4$ production but also enhance the ruminal efficiency of dietary nutrient use. It could be concluded from the previous studies that propolis, despite of its color or type can indirectly affect methane formation by their anti-protozoal effect (Soltan *et al*., 2014).

**Table 1. Effect of brazilian red propolis supplementation on feed intake, apparent diet digestibility and methane ($CH_4$) emission of ewes**

| Parameters | Treatments | | s.e.m | Pr > F |
|---|---|---|---|---|
| | Control | Propolis | | |
| **Daily dry matter intake [g/sheep]** | 1108 | 1229 | 55.3 | 0.279 |
| **Diet digestibility [%]** | | | | |
| Dry matter (DM) | 76.73 | 78.78 | 1.28 | 0.432 |
| Organic matter (OM) | 78.31$^b$ | 81.46$^a$ | 0.70 | 0.022 |
| Crude protein (CP) | 78.81$^b$ | 84.15$^a$ | 1.25 | 0.029 |
| Neutral detergent fibre (NDF) | 70.84 | 73.19 | 2.08 | 0.580 |
| Acid detergent fibre (ADF) | 49.13 | 73.19 | 2.83 | 0.650 |
| **Methane** | | | | |
| L/day | 25.21$^a$ | 22.63$^b$ | 0.66 | 0.047 |
| L/kg organic matter intake (OMI) | 25.01$^a$ | 22.72$^b$ | 0.52 | 0.023 |
| L/kg digestible organic matter (DOM) | 32.38$^a$ | 28.10$^b$ | 0.97 | 0.024 |

$^{a,b}$Means within a row without a common superscript letter differ significantly ($p < 0.05$).

Intake of nitrogen ($p < 0.05$) and body N retention ($p < 0.05$) expressed as g/animal/day were higher with BRP supplementation compared with the control diet (Table 2). Xanthine and hypoxanthine were not detected in urine while total purine derivatives (PD) tended to increase. The administration of BRP increased ($p < 0.05$) urinary allantoin derivative excretion compared to the control. Propolis was able to increase DMI, which reflected an increasing N intake. The increasing N body retention approved the ability of enhancing the ruminal degradation of nutrients by propolis, as reflected by the increase of the truly degraded OM and by the transfer of N to milk or meat production (Morsy *et al*., 2015). Microbial protein synthesis can be indicated by an increase in various indicators like the partitioning factor, the urinary PD excretion and the body N retention (Soltan *et al*., 2013). So the observed increase in body N retention, and the tendency of total PD and allantoin (which account for the major proportion in purine derivatives) excretion reveal increasing microbial protein synthesis with the propolis supplementation.

**Table 2. Effect of brazilian red propolis supplementation on nitrogen balance and urinary purine derivatives excretion of ewes**

| Parameters | Treatments | | s.e.m | Pr > F |
|---|---|---|---|---|
| | Control | Propolis | | |
| **Nitrogen balance** | | | | |
| Intake [g/d] | 37.0[b] | 40.7[a] | 0.86 | 0.032 |
| Faecal excretion [g/d] | 6.88 | 7.13 | 0.47 | 0.798 |
| Urinary excretion [g/d] | 7.96 | 6.59 | 0.67 | 0.317 |
| Body retention [g/d] | 19.5[b] | 22.9[a] | 0.71 | 0.017 |
| N retained [g/g N intake] | 0.52 | 0.64 | 0.03 | 0.073 |
| **Daily urinary excretion of purine derivatives (PD)** | | | | |
| Allantoin [mmol/animal] | 8.49[b] | 9.45[a] | 0.25 | 0.052 |
| Uric acid [mmol/animal] | 2.56 | 2.24 | 0.26 | 0.551 |
| Creatinin [mmol/animal] | 4.47 | 3.75 | 0.54 | 0.516 |
| Total PD [mmol/animal] | 10.73 | 11.82 | 0.32 | 0.095 |

[a,b]Means within a row without a common superscript letter differ significantly ($p < 0.05$).

**Table 3. Effect of brazilian red propolis supplementation on hematological, biochemical parameters and hormones of ewes**

| Parameters | Treatments | | s.e.m | Pr > F |
|---|---|---|---|---|
| | Control | Propolis | | |
| **Hematological parameters** | | | | |
| Erythrocyte (RBC 106/ml) | 10.96[b] | 12.14[a] | 0.30 | 0.056 |
| Total leukocyte (WBC 103/ml) | 11.03[b] | 13.36[a] | 0.52 | 0.007 |
| Hemoglobin (Hb g/dl) | 10.15[b] | 11.62[a] | 0.22 | 0.007 |
| Packed cell volume (PCV %) | 32. 53[b] | 34.61[a] | 0.51 | 0.040 |
| **Biochemical parameters** | | | | |
| Total protein (TP g/dl) | 7.31[b] | 8.95[a] | 0.11 | 0.001 |
| Albumin (Alb g/dl) | 2.83 | 2.74 | 0.03 | 0.227 |
| Globulin (Glo g/dl) | 4.48[b] | 5.22[a] | 0.12 | 0.001 |
| Glucose (Glu mg/dl) | 45.86[b] | 54.07[a] | 1.06 | 0.050 |
| **Hormones** | | | | |
| Cortisol (Cor µg/dL) | 1.98[a] | 1.16[b] | 0.140 | 0.002 |
| Triiodothyronin (T3 µg/dL) | 0.70[a] | 0.56[b] | 0.027 | 0.006 |
| Thyroxine (T4 µg/dL) | 7.81[a] | 6.31[b] | 0.335 | 0.022 |

[a,b]Means within a row without a common superscript letter differ significantly ($p < 0.05$).

The effect of BRP on hematological, biochemical parameters and hormones are presented in Table 3. Brazilian red propolis resulted in an increase (P<0.05) of all hematological parameters compared with control, moreover the same pattern was observed for some serum biochemical parameters such as total protein, globulin and glucose. Propolis supplementation resulted in a decrease (P<0.05) of cortisol, Triiodothyronin and Thyroxine compared to control. Recent studies have shown a general improvement of the animals health and immunity when treated by dietary of propolis (Talas and Gulhan 2009; Morsy *et al*., 2013).

## IV – Conclusions

Propolis, an alternative natural additive, increased diet digestibility, N intake and microbial N supply and decreased methane emissions. Propolis represents a promising natural antibiotic with profit biological and environmental effects during the late gestation of ruminants.


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