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Endogenous fraction and urinary recovery of purine derivatives in Nellore and Holstein heifers with abomasal purine infusion

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ABSTRACT

The excretion of purine derivatives (PD) as an index of microbial biomass production in the rumen can be biased by the endogenous fraction and the incomplete urinary recovery of absorbed purines. Moreover, several authors have suggested differences for endogenous fraction of purine derivatives between genetic groups. The objective of this study was to estimate the endogenous fraction of PD and the urinary recovery of purines in Nellore and Holstein heifers fed diets containing 60% corn silage and 40% concentrate daily (13 g dry matter/kg body weight). Daily creatinine excretion, intake and digestibility of dry matter and nutrients were also evaluated. Four Nellore heifers and four Holstein heifers fistulated at the rumen and abomasum, body weight of 270 ± 7.76 and 225 ± 7.16 kg, respectively, were allocated in two 4×4 Latin squares. The experimental treatments consisted of four doses of RNA infusion (*Torula Yeast*): 0, 33, 66, or 100 mmol/day. The endogenous losses of PD and the recovery of purine bases were estimated using a regression of the daily excretion of PD ($\hat{Y}$) and the abomasal flow of purine bases (X). There was no difference ($P > 0.05$) between the regression equations for PD excretion and daily abomasal flow of purines for each genetic group, resulting in the following regression: $\hat{Y} = 0.405 + 0.923X$, where 0.405 mmol/kg$^{0.75}$ and 0.923 represent the endogenous fraction and the recovery of purine bases in the abomasum, respectively, for both genetic groups. The infusion and genetic groups did not affect ($P > 0.05$) the daily creatinine excretion, which averaged 27.23 mg/kg BW. We concluded that there are no differences between Nellore and Holstein heifers in the endogenous fraction of PD, the urinary recovery of purines, and creatinine excretion, with average values of 0.405 mmol/kg$^{0.75}$, 0.92, and 27.23 mg/kg BW, respectively.

1. Introduction

Using the excretion of purine derivatives (PD) in ruminant animals as an index of microbial biomass production in the rumen was initially proposed by Blaxter and Martin (1962) and Topps and Elliott (1965). This measure was favored because of the need to develop noninvasive techniques for animal experimentation as an alternative to fistulated animals. Therefore, several authors demonstrated that the excretion of PD is directly related to purine absorption and that by knowing the relationship of N-purine:N-total microbial biomass, the production of microbial proteins can be calculated from the absorbed amount of purine, which is estimated by the urinary excretion of PD (Chen and Gomes, 1992; Fujihara et al., 1987; Orellana-Boero et al., 2001; Smith and McAllan, 1970). However, the relationship between the flow of RNA into the duodenum

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and the presence of PD in urine can be biased by the endogenous fraction and the incomplete urinary recovery of absorbed purines (Gonzalez-Ronquillo et al., 2003; Verbic et al., 1990). Generally, methods for estimating the endogenous excretion of purine involve the use of intragastric nutrition or prolonged fasting, which can alter the normal metabolism of the animal or the rate of degradation of the nucleic acids in tissues.

The endogenous fraction includes the proportion of PD excreted in urine that derives from nucleic acid degradation in animal tissues (Chen and Gomes, 1992). While Pimpa et al. (2001) stated that this fraction is similar between *Bos indicus* (Nellore) and *Bos taurus* (Holstein), other authors (Bowen et al., 2006; Chen and Gomes, 1992; Osuji et al., 1996) suggested there are genetic differences between groups. Bowen et al. (2006) worked with different genetic groups at the same physiological stage and experimental conditions. However the animals were fasted, which can alter the normal animal metabolism or the rate of degradation of the nucleic acids of tissues.

Regarding the urinary recovery of purines such as PD, some of the differences in the results can be explained by the presence of non-renal routes, including saliva and milk (Chen and Gomes, 1992), and by the difference in the digestibility of purines from ruminal microorganisms and purines from RNA-*Yeast* (Orellana-Boero et al., 2001). However, in cattle all of the hypoxanthine tends to be oxidized to uric acid. Whereas uric acid from this reaction cannot be used for *de novo* synthesis of purine (Chen and Gomes, 1992) and the major route for uric acid excretion is the urine (Mura et al., 1986), it would be expected that urinary recovery of infused purines would be high.

Therefore, this study was conducted to estimate the endogenous fraction and urinary recovery of purines, for Nellore and Holstein heifers fed at maintenance and receiving abomasal infusion of RNA as source of purine bases. Additionally, we evaluated the urinary creatinine excretion, intake, and the total and ruminal digestibility of the nutrients.

2. Materials and methods

2.1. Animals, treatment, and management

The experiment was conducted at the Animal Laboratory and the Laboratory of Ruminant Nutrition in the Department of Animal Sciences (DZO) of the Agrarian Science Center at the Federal University of Viçosa (Universidade Federal de Viçosa-UFV) in Viçosa, MG. All procedures were approved by the UFV Ethics Commission for Animal Use according to process 42/2010.

Eight heifers were used: four *B. indicus* (Nellore) and four *B. taurus* (Holstein), with initial body weights (BW) of 270 ± 7.76 and 225 ± 7.16 kg, respectively. They were fistulated in the rumen and abomasum according to techniques described by Leão and Coelho da Silva (1980) and confined at the Animal Laboratory of DZO/UFV. The animals were housed in covered, individual stalls that were made of concrete and covered with rubber. The stalls had an area of 9 m^2 and the heifers had free access to fresh water.

Table 1
Chemical composition of corn silage, concentrate and diet.

Item[a]	Silage	Concentrate[b]	Diet
DM[c]	299.9	872.8	529.0
OM[d]	957.2	964.6	960.1
CP[d]	58.3	180.8	107.3
EE[d]	26.2	19.1	23.3
NDFap[d]	477.2	11.7	333.2
NFC[d]	395.5	668.7	504.8

[a] DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF_{ap}: neutral detergent fiber: NFC: non-fibrous carbohydrates.
[b] Proportion of ingredients in concentrate (g/kg in fresh basis): corn meal, 762.8; soybean meal, 179.8; urea/AS, 12.4; ash, 27.5.
[c] g/kg fresh basis.
[d] g/kg DM in diet.

The diet was based on corn silage and concentrates at a 60:40 ratio on the dry matter (DM) basis. The animals were fed with 13 g DM/kg of BW daily (Table 1) in two similar amounts at 8h00 and 16h00. The diet was formulated to contain approximately 120 g/kg DM crude protein (CP), which is sufficient to meet maintenance requirements, according to BR CORTE (Valadares Filho et al., 2010).

The experimental treatments consisted of abomasal infusions of RNA (*Torula yeast*, type VI, Sigma®) at a rate of 0, 33, 66, or 100 mmol/day. All of the animals received the same diet beginning seven days before the first experimental period to allow for adaptation as well as throughout the entire experiment. The animals were assigned to treatments according to a 4×4 Latin square design (LS) that was balanced for residual effects, with four animals, four treatments, and four experimental periods. There was LS for each genetic group.

After diet adaptation by the heifers, each one of the four experimental periods was comprised of 14 day as follow: 1st to 5th day, adaptation; 6th to 9th day, abomasal digesta and feces collections at specific times; 9th to 13th day, abomasal infusion of RNA; 10th–13th day, total collection of urine and feces; 14th day, ruminal digesta collection. From the 1st to 9th day of each experimental period, each animal received 15 g of chromic oxide (Cr_2O_3) in a single dose through the ruminal fistula at 10h00 to obtain the DM flow in the abomasum.

From the 5th to 9th day, corn silage and concentrate supplied were sampled. At the end of each period, we obtained composite samples of the feeds, which were wrapped in plastic bags and stored at $-20\,^{\circ}C$ for later analyses.

The abomasal digesta and feces samples were taken at 15-h intervals (Allen and Linton, 2007) from at 7h00 on the 6th day through 10h00 on the 9th day. The samples were wrapped in plastic bags, pre-dried in a forced air oven at 60 °C for 72 h, and then ground in a Wiley Mill with a 1 mm sieve. A composite sample was made for each animal during the period based on the dry weight of the sample.

The RNA solutions, as a source of purines, were injected into the abomasum in daily amounts of 0, 33, 66, or 100 mmol. These quantities were divided into six equal doses given every four hours, beginning at 12h00 on

the 9th experimental day and ending at 8h00 on the 13th day. For the infusions, the abomasal cannulas were replaced by other cannulas fitted with polyethylene tube, approximately 15 cm in length, in the interior of the abomasum. For each infusion, the outer end of the polyethylene tube was connected to a manually controlled syringe. To ensure that the all of the RNA provided reached the abomasum and to remove residual RNA solution in the polyethylene tube, 50 mL of saline solution was injected at the end of each infusion.

The RNA solutions were always prepared one day before the infusion by diluting it in water alkalinized with NaOH (pH=11) at 40 °C (Orellana-Boero et al., 2001). After dilution, the pH was adjusted to 8 with concentrate HCl (Pimpa et al., 2001).

Total urine collection in each animal was obtained using a two-way, no. 22 Foley-type probes with a 30 mL balloon. At the free end of the probe, a polyethylene hose was attached through which the urine was conducted to plastic containers with lids. The containers were inserted into 80 L Styrofoam boxes, which were filled with ice to preserve creatinine (Van Niekerk et al., 1963). At the end of each 24-h period, the urine was weighed and homogenized. Next, we obtained a 10 mL sample that was diluted with 40 mL of 0.036 N H_2SO_4 and a 50 mL undiluted sample. These samples were stored at −20 °C for posterior PD (allantoin and uric acid) and creatinine analysis, respectively.

At 8h00 on the 14th day, we collected approximately 4 L of ruminal digesta from each animal through ruminal fistulas, which were used to isolate bacteria according to the technique described by Cecava et al. (1990).

2.2. Laboratory analyses

The composites of feed, feces, and refusals were analyzed for DM, crude protein (CP), and ash content, according the AOAC procedures (1990). Ether extract (EE) was measured by soxhlet extraction with petroleum ether. The neutral detergent fiber corrected for ash and protein (NDF_{ap}) was analyzed by adding heat-stable enzyme alpha-amylase (Ankon Tech. Corp., Fairport, NY) according to a technique adapted from Mertens (2003). Non-fibrous carbohydrates content corrected for ash and protein (NFC_{ap}) was calculated according to Detmann and Valadares Filho (2010), where $NFC_{ap}=100-[(\%\ CP-\%\ CP$ derived from urea+% urea)+% NDF_{ap}+% EE+% ash]. The total digestible nutrients (TDN) were calculated according to Weiss (1999) with the following equation: TDN (%)= dCP+dNDF+dNFC+2.25 dEE, where dCP=digestible crude protein, dNDF=digestible neutral detergent fiber, dNFC=digestible non-fibrous carbohydrates, and dEE= digestible ether extract. The chromium concentration in the abomasal digesta and feces samples was analyzed according to Williams et al. (1962).

The analysis of allantoin in the urine was carried out using the colorimetric method, following methods from Fujihara et al. (1987). The concentrations of uric acid were evaluated using an enzymatic colorimetric test with clearing factor lipase (Barham and Trinder, 1972; Fossati et al., 1980). The creatinine concentration was analyzed using the alkaline picrate method (Henry et al., 1974) in an AutoAnalyzer II (InVitro Diagnóstica Ltda, Itabira, MG, Brazil).

The purine bases were used to measure the production of microbial biomass that arrived at the abomasum as established by Ushida et al. (1985).

2.3. Calculations and statistical analyses

The abomasal flow of DM was obtained using the relationship between the given quantity of chromic oxide and the same concentration in the digesta samples. The total flow of purine bases in the abomasum was obtained by adding the value of purine bases of each animal before the infusion with the respective amount of purine bases infused. The amount of microbial nitrogen in the abomasum was calculated using the flow of N-RNA present in the abomasum divided by the N-RNA:N-total relationship from the bacteria isolated in the rumen.

The endogenous losses and recovery of purine bases as PD were estimated with a regression of the daily excretion of PD in the urine ($\hat{Y}$) and the amounts of purine bases in the abomasum (X), expressed in mmol/$kg^{0.75}$, which were represented by the intercept and regression coefficient (slope), respectively. The calculations were performed taking into consideration an experimental unit loss of a heifer that had a clinical finding of ruminal bloat.

The dependent variables were evaluated according to a 4×4 Latin square design, and the different genetic groups were organized in each of two squares using the model:

$$Y_{ijkl}=\mu+G_i+N_j+GN_{ij}+A_{(i)k}+P_{(i)l}+\varepsilon_{ijkl}$$

in which Y_{ijkl} is the dependent variable measured in animal k of genetic group i that was subjected to the j level of RNA infusion during period l, μ is the general constant, G_i is the effect of genetic group i confounded with the effect of Latin square i (fixed effect), N_j is the effect of abomasal infusion of RNAj (fixed effect), GN_{ij} is the effect of the interaction between genetic group i and the j level of infusion (fixed effect), $A_{(i)k}$=effect of animal k nested within to genetic group i (random effect), $P_{(i)l}$ is the effect of period l nested within Latin square i (random effect), and ε_{ijkl} is the random error assuming normal independent distribution (NID) (0; σ^2_ε).

Comparisons between the amounts of RNA infused were carried out through the orthogonal decomposition of the sum of squares associated with this source of variation in the linear, quadratic, and cubic effects. The analyses were conducted using PROC MIXED in SAS (*Statistical Analysis System*, version 9.1), assuming homogeneous variances between treatments and the degrees of freedom estimated by the Kenward–Roger method.

Comparisons between dependent variables on the genetic groups were made via the basic model:

$$Y_{ijkl}=\beta_0+\beta_1\times D+\beta_2\times X_{ijkl}+\beta_3\times D\times X_{ijkl}+A_{(i)k}+P_{(i)l}+\varepsilon_{ijkl}$$

in which Y_{ijkl} and X_{ijkl} are the variables that were taken into consideration to assess the relationship; D is the dummy variable to evaluate the effect of the genetic group on the relationship, with $D=0$ for Nellore heifers and $D=1$ for Holstein heifers; $A_{(i)k}$ and $P_{(i)l}$ are the random

effects previously described in the above model and used to adjust for experimental variation; and ε_{ijkl} is the random error assuming NID (0; σ^2_{ε}).

The regression models were adjusted according to the significance of the parameters β_1, β_2, and β_3 using the restricted maximum likelihood method implemented in PROC MIXED in SAS.

All of the statistical procedures were performed using 0.05 as the critical level for Type I error.

3. Results

There was no interaction ($P > 0.05$) between the genetic groups and the RNA infusion doses on the average daily intake (kg or g/kg BW) neither on the total and ruminal digestibilities. The infusion doses did not affect ($P > 0.05$) the intake of DM, OM, CP, EE, NDF_{AP}, NFC, and TDN (Table 2).

There was a significant difference between the genetic groups ($P < 0.05$) on the intake of DM, OM, CP, NDF_{AP}, and NFC, expressed in kg/day, with an average of 3.71, 3.56, 0.39, 1.25, and 1.85 for the Nellore heifers and 3.09, 2.97, 0.33, 1.04, and 1.54 for the Holstein heifers, respectively. When the intake of DM and NDF_{AP} were expressed as a function of BW, the genetic groups and infusion doses did not show significant effect ($P > 0.05$), with averages of 13.72 and 4.63 g DM/kg BW, respectively.

There was no effect ($P > 0.05$) from the infusion doses or genetic groups on the total digestibility of DM, OM, EE, NDF_{AP}, NFC, and on the levels of TDN, which had averages of 728.1, 744.9, 847.1, 601.0, 859.4, and 743.6 g/kg DM, respectively. The total digestibility of CP was not affected by the genetic group ($P > 0.05$) but did show a significant linear effect ($P < 0.05$) with the level of infusion.

There was no effect ($P > 0.05$) from the infusion doses and genetic groups on the ruminal digestibillity of DM, OM, CP, EE, NDF_{AP}, and NCF, which had averages of 624.0, 668.5, 211.1, 134.4, 903.0, and 629.6 g/kg DM, respectively.

There was no difference ($P > 0.05$) between the genetic groups in the composition of the ruminal bacteria. The average values of RNA, N-RNA, N-total, and the N-RNA:N-total ratio, expressed on the DM basis, were 4.60, 0.67, 6.10, and 0.11, respectively (Table 3). The abomasal flow of RNA, expressed in g/day, was not different ($P > 0.05$) between the genetic groups, with a daily average of 38.08 g.

Relating the excretion of PD ($\hat{Y}$) and the daily abomasal flow of purines (X), expressed in mmol/$kg^{0.75}$, there was no difference ($P > 0.05$) on the intercept or the slope

Table 2
Effects of genetic group and RNA abomasal infusion on nutrient intake and digestion.

Item[a]	Genetic group								SEM[a]	*P*-value[*]		
	Nellore				Holstein					GG[a]	Differences	
	Level of Infusion (mmol/day)										L[a]	Q[a]
	0	33	66	100	0	33	66	100				
Intake (kg/day)												
DM	3.72	3.69	3.70	3.74	3.08	3.10	3.09	3.09	0.18	0.046	0.486	0.545
OM	3.57	3.54	3.55	3.59	2.96	2.98	2.97	2.98	0.17	0.046	0.460	0.479
CP	0.39	0.39	0.39	0.40	0.33	0.33	0.33	0.33	0.02	0.046	0.531	0.356
EE	0.085	0.090	0.087	0.073	0.073	0.073	0.073	0.073	0.006	0,084	0,147	0,168
NDF_{ap}	1.25	1.24	1.24	1.25	1.03	1.04	1.04	1.04	0.06	0.049	0.526	0.479
NFC	1.85	1.84	1.84	1.87	1.53	1.55	1.54	1.54	0.08	0.046	0.387	0.516
TDN	2.82	2.74	2.64	2.82	2.33	2.34	2.31	2.26	0.16	0.093	0.211	0.142
Intake (g/kg BW)												
DM	13.7	13.7	13.7	13.8	13.7	13.7	13.7	13.8	0.03	0.981	0.139	0.098
NDF_{ap}	4.63	4.63	4.63	4.63	4.63	4.63	4.63	4.63	0.16	0.998	0.397	0.525
Total tract digestion (g/kg DM)												
DM	742.9	726.3	697.1	736.3	743.6	740.8	733.2	707.9	1.82	0.796	0.052	0.350
OM	755.1	743.9	713.6	752.1	759.7	757.8	749.7	727.6	1.78	0.724	0.083	0.413
CP	672.0	666.1	617.9	655.4	657.4	648.8	630.0	607.0	2.35	0.559	0.016	0.515
EE	853.8	833.5	839.8	835.2	855.0	857.8	876.3	825.7	1.32	0.379	0.097	0.234
NDF_{ap}	613.6	599.1	584.6	611.3	613.7	615.8	582.4	587.7	1.70	0.900	0.161	0.295
NFC	865.1	855.3	816.6	864.9	876.7	873.6	878.8	844.5	2.24	0.429	0.325	0.652
TDN	753.6	742.2	713.2	750.1	757.9	756.2	749.2	726.3	1.75	0.721	0.078	0.436
Ruminal digestion (g/kg DM)												
DM[b]	603.2	479.2	650.0	572.3	686.7	653.7	647.8	699.1	7.10	0.251	0.758	0.424
OM[b]	651.0	534.2	690.0	627.2	724.2	693.3	685.2	745.2	6.59	0.269	0.688	0.347
CP[c]	202.4	114.0	229.5	238.5	286.1	196.8	210.1	211.5	7.12	0.718	0.967	0.226
EE[c]	215.9	−26.1	80.3	130.0	149.7	155.8	154.0	215.2	10.32	0.596	0.917	0.093
NDF^{b}_{ap}	918.6	837.2	951.8	884.6	913.7	901.4	901.4	909.6	3.82	0.809	0.979	0.753
NFC[b]	590.4	471.8	624.6	566.5	706.4	661.9	665.3	750.1	8.91	0.236	0.596	0.313

[*] No effect ($P > 0.05$) for cubic effect and the interaction QL*Treatment.

[a] DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF_{ap}: neutral detergent fiber corrected for ash and protein; NFC: non-fibrous carbohydrates; TDN: total digestible nutrients (kg/day); SE: standard error of the; GG: genetic group; L: linear; Q: quadratic;

[b] % of total tract digestion.

[c] % of nutrient intake.

Table 3
Bacterial composition (%DM) in Nellore and Holstein heifers.

	Genetic Groups	
	Nellore	Holstein
RNA	$5.02_{\pm 1.61}$	$4.45_{\pm 1.60}$
N-RNA	$0.73_{\pm 0.23}$	$0.64_{\pm 0.23}$
N-total	$6.65_{\pm 1.56}$	$5.72_{\pm 1.21}$
N-RNA:N-total	$0.109_{\pm 0.02}$	$0.111_{\pm 0.03}$

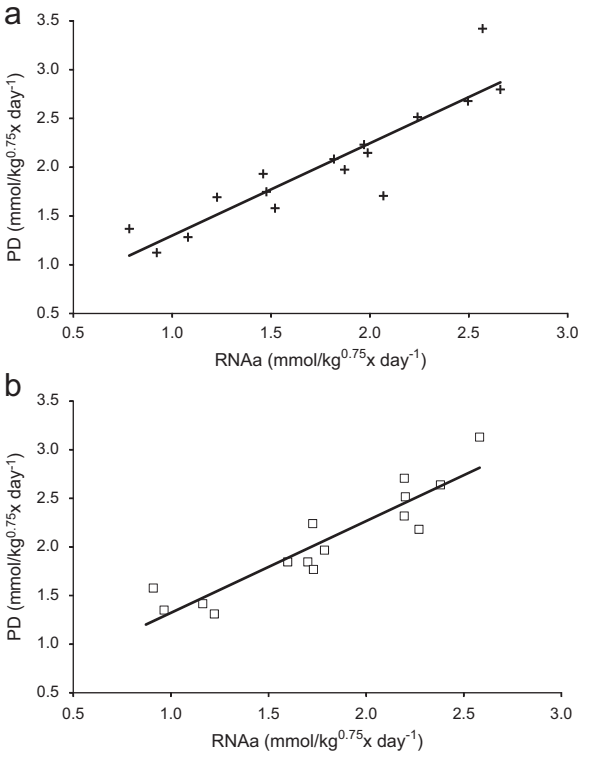

Fig. 1. Purine derivatives excretion (PD, Y) in the urine of Nellore (a) and Holstein (b) heifers as a function of the daily RNA abomasal flow (RNAa, X): [$\hat{Y}=0.389_{\pm 0.201}+0.051_{\pm 0.308}\times D+0.926_{\pm 0.105}\times X-0.014_{\pm 0.160}\times(D\times X)$]; where $D=0$ for Nellore and $D=1$ for Holstein; $s_{XY}=0.2212$.

between the genetic groups, when considering the following regression equation: $\hat{Y}=0.389_{\pm 0.201}+0.051_{\pm 0.308}\times D+0.926_{\pm 0.105}\times X-0.014_{\pm 0.160}\times(D\times X)$, in which $D=0$ for Nellore and $D=1$ for Holstein and $s_{XY}=0.2212$ (Fig. 1). When $D=0$ for Nellore heifers, the resulting equation was $\hat{Y}=0.389+0.926X$, where the endogenous fraction of PD was 0.389 mmol/kg$^{0.75}$ and the urinary recovery of purines was 0.926 (Fig. 1a). When $D=1$ for Holstein heifers, we obtained the equation $\hat{Y}=0.439+0.911X$, indicating a urinary recovery of 0.911 and an endogenous fraction of 0.439 mmol/kg$^{0.75}$ (Fig. 1b).

There was no difference ($P>0.05$) between the regression equations for PD excretion and daily abomasal flow of purines for each genetic group, which suggests that there is no difference between Nellore and Holstein heifers when they are subjected to the same experimental and feeding conditions. In this manner, the following regression equation was generated for both: $\hat{Y}=0.405_{\pm 0.148}+0.923_{\pm 0.077}\times X$; $_{sXY=0.219}$ (Fig. 2), where 0.405 mmol/kg$^{0.75}$ and 0.923 represent the endogenous fraction and the recovery of purine bases in the abomasum, respectively, for both genetic groups.

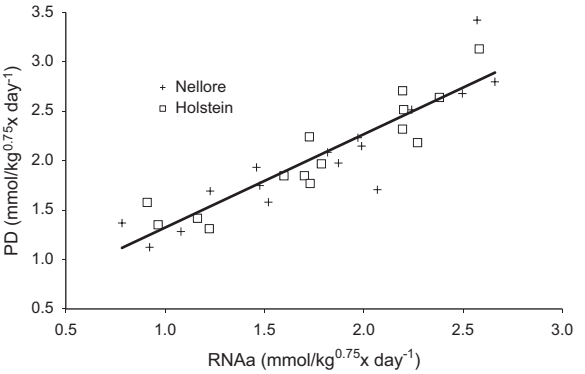

Fig. 2. Purine derivatives excretion (PD, *Y*) in the urine of Nellore and Holstein heifers as a function of the daily RNA abomasal flow (RNAa, *X*): ($\hat{Y}=0.405_{\pm 0.148}+0.923_{\pm 0.077}\times X$; $_{sXY=0.219}$).

There was no interaction ($P>0.05$) or individual effect ($P>0.05$) of the genetic group or the level of abomasal infusion of RNA on the daily urinary excretion of creatinine, which averaged 27.23 mg/kg BW (Table 4).

4. Discussion

The significant effect from the genetic group on intake was probably due to the average BW, which was 270 ± 7.76 kg for Nellore heifers and 225 ± 7.16 kg for Holstein heifers. Accordingly, when the DM and NDF_{AP} intake were expressed as a function of BW, we did not observe an effect from the genetic group. Notably, the level of intake was planned previously. As Orellana-Boero et al. (2001) and Barbosa et al. (2011) suggested, keeping the intake at a maintenance level is a procedure that approximates to the normal conditions of feeding, without promote metabolic changes in the animal, particularly changes in the degradation rate of nucleic acids (Chen and Ørskov, 2003).

Digestibility is related to intake and the type of diet (Van Soest, 1994). Because the animals were fed with only the provided diet of 13 g DM/kg of BW, variations in digestibility were not expected. The effect of the infusion of purine bases on the total digestibility of CP was reported by Orellana-Boero et al. (2001), but it was not confirmed by other authors (Ojeda et al., 2005; Pimpa et al., 2001).

Feeding the animals a single diet in proportion to their BW can also explain the bacterial composition results, as it is directly related to the type and quantity of feed intake (Maynard et al., 1979).

Regarding the effect of genetic groups, Bowen et al. (2006) found difference ($P<0.001$) for endogenous losses of PD 0.414 and 0.1898 mmol/kgPC$^{0.75}$, respectively, in *B. taurus* and *B. indicus* fasted for 7 days. However, the same authors did not find difference ($P=0.2$) for the average excretion of PD during the trial period in which the

Table 4
Effects of genetic group and RNA abomasal infusion on daily urinary creatinine excretion.

Item	Genetic groups								SEM[b]	*P*-value[a]		
	Nellore				Holstein					GG[b]	Difference	
	Level of Infusion (mmol/day)										*L*[b]	*Q*[b]
	0	33	66	100	0	33	66	100				
Creatinine mg/kg BW	27.67	27.39	27.18	27.37	27.09	27.41	27.18	26.52	0.544	0.714	0.380	0.343

[a] No effect ($P > 0.05$) for cubic effect and the interaction QL*Trat.
[b] SEM: standard error of the mean; GG: genetic group; *L*: linear; *Q*: quadratic.

animals were fed at maintenance. Bowen et al. (2006) stated that the reason for the low endogenous excretion PD in *B. indicus* cattle is unclear. Osuji et al. (1996) obtained values numerically lower of endogenous losses of DP for crossbred (*B. indicus* × *B. taurus*) when compared with Zebu (*B. indicus*), respectively, 0.108 and 0.172 mmol/kg $PC^{0.75}$ for animals fasted for 20 days. The authors suggested that there were differences in the N use between Zebu and their crosses. However they did not explain why crossed animals have endogenous loss lesser than Zebu.

The values of the endogenous fraction according to Chen and Ørskov (2003) should be 0.147 and 0.385 mmol/$kg^{0.75}$ for zebu and taurine animals, respectively. However, this recommendation was based on values obtained from experiments performed using different techniques, animals in different physiological stages, and different gender. The value of the endogenous fraction of PD found in the literature for *B. indicus* and *B. taurus* animals varied from 0.146 to 0.350 mmol/$kg^{0.75}$ and from 0.236 (Orellana-Boero et al., 2001) to 0.531 mmol/$kg^{0.75}$ (Beckers and Thewis, 1994), respectively. These values are within the confidence interval (CI) determined in this experiment, which was [CI $(\beta_0)_{0.95}$: $(0.101 \leq \beta_0 \leq 0.709)$], where β_0 is the parameter for endogenous fraction. This variation can be explained by the different techniques and genders used. Orellana-Boero et al. (2001) worked with Holstein dry cows and used the isotope dilution technique (N^{15}) for infused bases, while Beckers and Thewis (1994) used Belgian Blue bulls. Moreover, Barbosa et al. (2011) described the value of 0.301 mmol/kg $BW^{0.75}$ for endogenous losses for *B. indicus* heifers using abomasal infusion of purines. Although numerically different, the value is included in the CI of the present experiment. This probably can be attributed in part to the large animal variation (Chen et al., 1990; Verbic et al., 1990).

The confidence interval for the urinary recovery of purine bases infused in the abomasum (β_1) was [CI $(\beta_1)_{0,95}$: $(0.760 \leq \beta_1 \leq 1.086)$]. Several values found in the literature, such as 0.77 (Verbic et al., 1990), 0.86 (Vagnoni et al., 1997), 0.85 (Pimpa et al., 2001), and 0.80 (Barbosa et al., 2011), are within this CI. However, other authors reported lower levels, such as 0.72 (Beckers and Thewis, 1994), 0.68 (Orellana–Boero et al. (2001)), and 0.58 (Gonzalez-Ronquillo et al., 2003).

Prasitkusol et al. (2002) suggested that the wide variation in urinary recovery of PD in different experiments can be attributed to the different techniques used, but they reinforced that these sources of variation need to be identified for better precision in the duodenal flow of purines. Chen and Gomes (1992) indicated that PD could be excreted via non-renal routes through secretion in the gastrointestinal tract via saliva or the intestinal wall and secretion in milk. However, they did not identify the real contribution of each of these routes.

The main PD in cattle urine were allantoin and uric acid (Chen and Gomes, 1992; Chen et al., 1990; Fujihara et al., 1987). Mura et al. (1986) observed, using the activity of xanthine oxidase present in the liver of zebu, that virtually all hypoxanthine was oxidized to uric acid, which could not be reused for *de novo* synthesis of purines (Chen and Gomes, 1992). Therefore, the values for urinary recovery of PD obtained in this work are probably more suitable for cattle, as urine is the major form of excretion of these PD (Chen and Gomes, 1992). However, more studies should be performed to evaluate the real contribution of renal and non-renal routes for PD excretion.

The recovery of absorbed purines (AP) can be obtained by assuming that the true digestibility of purines (TDP) is 0.93 according to Barbosa et al. (2011). In this case, dividing the obtained urinary recovery of PD (0.923) by 0.93 would yield a recovery of AP of 0.99. Therefore, the AP can be estimated from the urinary PD by using the following equation: AP = PD − (0.405 mmol/$kg^{0.75}$) absorbed purines (AP)/0.99. The production of microbial nitrogen (Nmic) can be estimated from the AP using the model proposed by Chen and Gomes (1992): Nmic (gN/day) = 70 AP/(TDP × NRNA:Ntotal × 1000), which results in the following equation: Nmic = 70 AP/(0.93 × 0.11 × 1000), with a TDP value of 0.93 in the small intestine (Barbosa et al., 2011) and a ratio N-RNA:N-total of 0.11 from this experiment.

For the animals submitted to infusion zero of RNA the values of absorbed purines and Nmic production were calculated as proposed by several authors (Table 5). The endogenous loss of PD proposed by Bowen et al. (2006) was replaced in the equation of Chen and Ørskov (2003).

There are numerical similarities between the purine absorbed observed in this study (113.36 ± 10.83) and by Barbosa et al. (2011), 116.11 ± 14.89. For Nmic production the difference of 18% between estimates can be

Table 5
Absorbed purines (AP) and production of microbial nitrogen (Nmic) obtained for *Bos taurus* and *Bos indicus* from equations proposed by several authors and by this study.

Absorbed purines (mmol/kg$^{0.75}$)		Nmic (g/day)		Authors
Equations	Values	Equations	Values	
B. taurus				
AP = PD − (0.439 mmol/kg$^{0.75}$)/0.99	110.90 ± 7.25	Nmic = 70 AP/(0.93 × 0.11 × 1000)	75.24 ± 4.92	This study
AP = PD − (0.385 mmol/kg$^{0.75}$)/0.85	110.35 ± 7.25	Nmic = 70 AP/(0.83 × 0.116 × 1000)	80.23 ± 5.27	Chen and Ørskov (2003)
AP = PD − (0.414 mmol/kg$^{0.75}$)/0.85	108.38 ± 7.26	Nmic = 70 AP/(0.83 × 0.116 × 1000)	78.80 ± 5.28	Bowen et al. (2006)
B. indicus				
AP = PD − (0.389 mmol/kg$^{0.75}$)/0.99	114.92 ± 14.91	Nmic = 70 AP/(0.93 × 0.11 × 1000)	78.64 ± 10.21	This study
AP = PD − (0.147 mmol/kg$^{0.75}$)/0.85	129.56 ± 15.89	Nmic = 70 AP/(0.83 × 0.116 × 1000)	94.2 ± 11.6	Chen and Ørskov (2003)
AP = PD − (0.30 mmol/kg$^{0.75}$)/0.80	116.11 ± 14.89	Nmic = 70 AP/(0.93 × 0.137 × 1000)	63.79 ± 8.24	Barbosa et al. (2011)
AP = PD − (0.19 mmol/kg$^{0.75}$)/0.85	126.20 ± 15.66	Nmic = 70 AP/(0.83 × 0.116 × 1000)	91.75 ± 11.39	Bowen et al. (2006)
B. taurus and *B. indicus*				
AP = PD − (0.405 mmol/kg$^{0.75}$)/0.99	113.36 ± 10.83	Nmic = 70 AP/(0.93 × 0.11 × 1000)	77.57 ± 7.41	This study

assigned to the different composition of bacteria observed in both studies.

Regarding *B. taurus*, there are numerical similarity among the Nmic production observed in this study, by Chen and Ørskov (2003) and by Bowen et al. (2006), 77.57, 80.23, and 78.80, respectively. In relation to *B. indicus*, there is approximately 14% of variation in the estimates of absorbed purines. Other 20% variation in the estimates of Nmic production could be assigned to the wide variation in endogenous PD excretion, the differences between N content in bacteria, the differences in urinary recovery of purine and the true digestibility of purine.

The confidence interval for the daily excretion of creatinine (β_3) was [CI(β_3)$_{0.95}$: ($35.11 \leq \beta_3 \leq 19.36$)], comprising the values found in the literature of 27.36 mg/kg BW (Rennó et al., 2000) and of 26.35 mg/kg BW (Pereira, 2009). Daily excretion of creatinine was not affected by RNA infusion into the abomasum and the genetic groups. This can be related to the use of creatinine in *spot* samples. In this experiment would not be expected large differences in creatinine excretion once intake was fixed. However in field application, intake is not controlled such as in this experiment; hence there needs to be caution in application of the method. Vagnoni and Broderick (1997) reported a significant 4% increase in creatinine excretion when high moisture ear corn was increased from 24% to 40% of dietary DM in cows fed alfafa silage or hay as the sole forage. Nevertheless, there were no overall differences due to concentrate level or sampling interval on creatinine clearance (Valadares et al., 1999) in lactating Holstein cows, reinforcing the idea that BW is the main determinant of creatinine excretion (Chizzotti et al., 2008; Pereira, 2009) and that urinary creatinine excretion can be used as a marker of daily urinary production (Valadares et al., 1997).

5. Conclusion and recommendations

The lack of difference between genetic groups enables use the same endogenous purine fraction and urinary recovery, 0.405 mmol/kg$^{0.75}$ and 0.92, respectively, for Nellore and Holstein heifers. The creatinine daily excretion is 27.23 mg/kg BW regardless genetic groups.

Conflict of interest statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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