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ESTIMATION OF MICROBIAL PROTEIN SYNTHESIS FROM DERIVATIVES OF PURINE IN CATTLE AND BUFFALOES THAT WERE FED WITH INCREASED LEVELS OF CONCENTRATE DIETS

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ABSTRACT

This work aims to estimate the synthesis of microbial protein and urinary creatinine excretion in four bovine and four bubaline distributed in two 4 × 4 Latin squares. The treatments consisted of different levels of concentrate (0.0, 24.0, 48.0, and 72.0%) composed of grinded (SIM) corn, soybean meal, mineral salt, and Tifton-grass hay. Each period lasted 21 days; 14 days of adaptation and seven days to collect samples. The results presented interaction between species for the urinary volume, uric acid, allantoin, purine derivatives, absorbed purines, microbial nitrogen, microbial crude protein, microbial crude protein efficiency of synthesis microbial, urinary creatinine, for interaction with plasma creatinine and creatinine clearance. Increased levels of concentrate in the diet did not influence the urinary volume, efficiency of microbial protein synthesis, plasma creatinine and creatinine excretion in buffaloes. For the observed concentrate levels' effect for purine derivatives and microbial protein production, as well as for the plasma creatinine and creatinine excretion in cattle, rising levels of concentrate in the diets of cattle and buffaloes provide increases in both purine derivatives and microbial protein production; the collection of spot urine is a tool to estimate purine derivatives excretion and creatinine, to estimate the urinary volume in animals.

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INTRODUCTION

The researchers have discussed about metabolic changes in ruminants and their effects on animal performance, as well as metabolic changes between the bovine and buffaloes, however bubaline species present greater need for more accurate understanding in terms of scientific results about their metabolism and productive chain. Microbial nitrogen (N) depends on the available energy in the rumen. One of the major protein sources for these animals has microbial origin which is synthesized from the rumen fermentation process through degradation of dietary food. It is important to consider that increased microbial protein which is synthesized in the rumen increases protein intake in a good amino acid level in the small intestine and it contributes to a decreasing in N-ammonia concentration in the rumen (Calsamiglia et al., 2010). Food consumption is the main factor that determines the supply of nutrients to meet the requirements for maintenance and production animal (Chizzotti, 2007). According to the NRC (1996), the main sources of amino acids for ruminants come from the true digestible microbial protein (PMIVD) and non-degraded dietary protein in the rumen which is digested in the small intestine (PNDRD).

Microbial protein may supply more than 50% of the amino acids available for absorption in the small intestine and it is considered high quality protein source once that it presents a high intestinal digestibility and its amino acid level closely resembles tissue and milk protein ones (Schwab, 1996). Therefore, there is a need to intensify studies on the metabolism of rumen microorganisms in order to obtain information that will allow better understanding of physiological chemical process of microbial protein production, as well as the effects related to production levels.
The dietary amount not degraded in the rumen that reaches the small intestine depends on the ruminal degradation which is influenced by the type of diet. This study aimed to estimate the microbial protein synthesis by non-invasive methods through the excretion of purine derivatives in urine and to estimate the urinary creatinine excretion in cattle and buffaloes that were fed increasing levels of concentrate.

MATERIALS AND METHODS

The experiment was conducted in the Animal Science Department of Universidade Federal Rural de Pernambuco. Four cattle 5/8 zebu dutch, and four Murrah buffaloes with an average weight of 461.19 ± 7.59 and 455.44 ± 7.85 kg for cattle and buffaloes respectively, were used. They were initially submitted to endo and ectoparasite control and fed with increased levels of concentrate diets (0.0, 24.0, 48.0, and 72.0%) based on dry matter (DM). The roughage used was composed of Tifton hay (Cynodonspp) and the concentrate of ground corn, soybean meal, and mineral salt. The diets were formulated according to the NRC (1996). The animals were housed in individual pens and they were fed twice a day allowing remains of 10% of the dry matter. The experimental design was Latin square 4 X 4 for each specie with four periods, four diets, and four animals. The experiment lasted 84 days divided on periods of 21 days, 14 days of animal adaptation to the diets and management and seven days for collection of data and samples. Table 1 presents the chemical compositions of the diets ingredients. The animals were weighed in the first and last days of collection of each experimental period aiming to observe weight changes after 16 hours of fasting. Excreta samples were collected in the morning and afternoon, and then it was obtained composed samples per animal per period were. This material was pre-dried at 55°C and grounded in a mill with a 1 mm sieve for bromatological analyses and in a 2mm sieve to estimate fecal dry matter production. Table 1 presents bromatological compositions of the diets' ingredients. Table 2 shows the proportions of ingredients in the experimental diets, as well as the chemical composition of experimental diets in DM basis. The "spot" urine collection was performed on the last day of each collection period, four hours after the food supply and during spontaneous urination. The urine was stored in a container of 100 ml capacity. Then, an aliquot of 10 ml was collected which was immediately diluted in 40 mL of H2SO4 (0,036N). The pH was adjusted to below 3 drops of concentrated H2SO4 to prevent bacterial destruction of the purine derivatives and uric acid precipitation. The samples were frozen at -20°C for subsequent analysis of creatinine, allantoin, and uric acid. Simultaneously blood samples were collected from each animal by jugular vein puncturing, using 10 ml "Vacutainer" pipes and heparin as an anti-coagulant. The samples were centrifuged at 2,000 rpm for 15 minutes. The resulting plasma was placed in "Eppendorf" tubes and it was frozen at -20°C for creatinine levels analysis. The uric acid in urine and plasma and the (Doles) ® commercial kit were used while following the manufacturer's technical guidelines for the analysis of creatinine. The analysis of allantoin was performed according to Chen and Gomes (1992). Urinary volume was estimated for each animal by multiplying body weight (BW), daily creatinine excretion (mg/kg BW) and (mmol/kg0,75), while dividing the product by the concentration of creatinine (mg/L) and (mmol/L) in the urine, for cattle and buffaloes respectively. The daily creatinine excretion was obtained by adopting the average of 27,76 mg/kg BW which was obtained by Renno (2003) for cattle and buffaloes and the average value of 0,44 mmol/kg0,75 which was reported by Chen et al. (1996).

The total excretion of purine derivatives (DP) was calculated by adding uric acid excretion in the urine and the amount of allantoin in the urine expressed the result in mmol/day. For cattle, the absorbed purines (PA) (X, mmol/day) were calculated from the excretion of DP (Y, mmol/day) through the following equation: X = {Y – (0.385 * PV0,75)} / 0.85, where 0.85 is the recovery of purines absorbed as DP, 0.385 is the endogenouscontribution to the excretion of purines, and $PV^{0,75}$ is the metabolic body weight (Verbic et al., 1990). The equation X = {Y – (0,117 * $PV^{0,75}$) / 0,74 was used for buffalos, as recommended for Dipu (2006). The microbial nitrogen synthesis (SNmic) for buffaloes (Y, gN/day) was calculated according to the PA (X, mmol/day) by the formula Y = 70X / 0.83 x 0.116 x 1000, in which 70 is the purine nitrogen (mg/mol), 0.83 is microbial purine digestibility, and 0.116 is the N purine ratio:N total bacteria as described by Chen and Gomes (1992). The value 0.116 was modified to 0.134, for cattle, according to Valadares et al. (1999). This change resulted in the equation Y = 70X / 0.83 x 0.134 x 1000. The estimation of microbial crude protein (PBmic) was calculated by calculating the SNmic x 6.25 and the efficiency of protein synthesis was determined from the microbial crude formula: ESPBmic (g/kg) = PBmic (g) / CNDT (kg) where CNDT = total digestible nutrient consumption. The results were analyzed statistically by analysis of variance and regression using the SAS (2011), considering 5% of significance by the F test. To check the existence of an interaction between results for cattle and buffaloes, the analysis of variance was performed and it was observed interaction between four levels of concentrate and the two Latin squares (QL). A response between different species was considered in case of interaction. Therefore, the analysis of variance was performed individually for each species (16 observations for cattle and 16 observations for buffaloes) to verify which model that fits on the data. However, when there was a lack of interaction this procedure was performed by computing the 32 pieces of data together. The effects of concentrate levels on the studied variables were evaluated after regression analysis, thereby proceeding the data from each LS, or both LS, individually or gathered according to the verified interaction. The significance of the regression coefficients was observed using the F test at 5% which was adjusted by dividing the mean square of the chosen model and the mean square referring to the 32 observations. The criteria that was used to select the model was based on the significance of the regression coefficients, the coefficient of determination (R2), as well as the biological phenomenon.

RESULTS

In the present study, an interaction was not observed between species and the urinary volume VU(L) AU uric acid (mmol/d),

Table 1. Chemical composition (g/kg) of the diet ingredients

Items	Feed		
	Hay Tifton	Ground corn	Soybeanmeal
Dry matter[a]	881.1	885.5	882.4
Organic matter	919.6	978.9	916.8
Crude protein	80.2	99.2	500.0
Neutral detergent fiber[b]	759.5	141.7	151.5
Acid detergent fiber[b]	443.4	33.3	37.3
Ether extract	25.6	48.7	33.4
Mineral matter	80.4	21.1	83.2
Mineral matter	813.7	831.1	383.4
Non-fiber carboydrates	54.2	677.9	231.0

[a]g/kg of fresh matter; [b]Corrected to ash and protein.

Table 2. Ingredient proportions and chemical composition of the experimental diets (g/kg)

Items	Concentrate levels in the diet			
	0.0	24.0	48.0	72.0
	Ingredients (% DM of the diet)			
Hay Tífton	100,0	76,0	52,0	28,0
Ground corn	0,0	17,76	35,52	53,28
Soybeanmeal	0,0	6,24	12,48	18,72
Nutrients	Composition of the diets			
DM (g/Kg NM)	88,11	88,20	88,28	88,37
Organic matter	91,96	92,99	94,03	95,07
Crude protein	8,02	10,98	13,94	16,89
Neutral detergent fiber[b]	75,95	61,18	46,42	31,65
Acid detergent fiber[b]	44,34	34,52	24,71	14,89
Ethereal extract	2,56	3,02	3,48	3,93
Mineral matter	8,04	7,01	5,97	4,93
Total carboydrate	81,37	78,99	76,62	74,24
Non fiber carboydrate	5,42	17,81	30,20	42.59
Total digestible nutrients[c]	52,28	58,74	67,32	64,38
Total digestible nutrients[d]	53,35	61,62	69,14	77,04

[1], SNIFFEN et al. (1992).
[2], NRC (2001).

Table 3. Means,coefficient of variation(CV), determination coefficient (R^2), regressionequation(ER), urine volume, purine derivatives,andmicrobial proteinin cattle andbuffaloes that were fed with increased levels of concentrate diets

	Increasing levels of concentrate				CV	R^2	ER
	0.0	24.0	48.0	72.0			
UV (L)	14.9	16.3	18.0	18.6	24.8	-	$\hat{Y}$ = 16.9
AL (mmol/d)	87.3	109	126	141	22.2	0.99	$\hat{Y}$ = 89.083 + 0.7412**x
UA (mmol/d)	15.1	21.7	31.9	32.4	42.4	0.91	$\hat{Y}$ = 15.995 + 0.258**x
PD (mmol/d)	102	131	158	173	23.7	0.98	$\hat{Y}$ = 105.08 + 0.9992**x
PABS(mmol/d)	96.2	130	164	184	28.5	0.99	$\hat{Y}$ = 98.936 + 1.2347**x
micN (g/d)	64.8	96.8	110	123,51	28,4	0,99	$\hat{Y}$=66,336+0,83**x
CPmic (g/d)	405	542	686	771,93	28,4	0,99	$\hat{Y}$=414,6+5,1873**x
micCPSE	125	111	103	106,14	34,6	-	$\hat{Y}$=111,35
CPImic (g/kg ICB)	1161	84	671	716,51	41,2	0,77	$\hat{Y}$=1072,8-6,2655**x

Table 4. Plasmaand urinaryconcentrations ofcreatininein cattle andbuffaloes that were fed with increased levels of concentrate diets

	Increasing levels of concentrate				CV	R^2	ER
	0,0	24,0	48,0	72,0			
	Cattle						
CP (mg/dl)	1,66	1,40	1,37	1,14	12,7	0,93	$\hat{Y}$=1,629-0,006**x
CC (ml/min)	551,04	688,04	716,52	859,60	16,0	0,95	$\hat{Y}$=560,6+3,975**x
	Buffaloes						
PC (mg/dl)	1,59	1,70	1,66	1,88	15,2	0,81	$\hat{Y}$=1,71
CC (ml/min)	1168,48	1101,40	1157,85	1029,18	11,0	-	$\hat{Y}$=1114,23
UCr (mg/dl)	156,21	141,97	141,68	139,28	25,8	-	$\hat{Y}$=144,78
UCr (mg/kg^{0,75})	194,65	194,84	195,25	194,91	1,2	-	$\hat{Y}$=194,91
CE (g/d)	19,95	20,05	20,21	20,10	4,7	-	$\hat{Y}$=20,08

**Significant at5% probability, by F test; Coefficient of variation(CV), regressionequation(ER), determination coefficient (R^2), plasmacreatinine (PC), creatinineclearance (CC), urinary creatinine(UCr), and creatinine excretion(CE).

allantoin AL (mmol/d), purine derivatives DP (mmol/d), absorbed purines PABS (mmol/d), microbial nitrogen Nmic (g/d), microbial crude protein PBmic (g/d), microbial crude protein synthesis efficiency ESPBmic, and microbial protein by crude protein intake PMIC (g/kg CPB), urinary creatinine in (g/d) (mg/PV0,75), with an interaction for plasma creatinine (CP) and creatinine excretion (CC). In Table 3, it was observed no effect of concentrate levels for urinary volume analyzed as one, as determined by the creatinine indicator. For AL concentrations (mmol/d), an influence of concentrate levels was observed related to linear behavior increasing; a mean of 115.77 mmol/ay was obtained, with 82% of total

purine derivatives found in urine. The AU (mmol/d) was also influenced by the concentrate levels in the diet, demonstrating a linear increase with an average of 25.35 mmol/d with a percentage of 17.58% of the total excreted purine derivatives. The same behavior was observed with DP (mmol/day), which increased linearly with the inclusion of concentrate in the diets. The data that was obtained for the two species together had a mean of 141,05mmol/d. Concentrate levels caused significant effects on the PABS, presented in a linear increased average 143,38mmol/d. Nmic (g/d) affected concentrate levels, providing an increased linear behavior.

There was no interaction between animal species and the influence of concentrate levels for ESPBmic, despite the numerical differences, averaged 111,35 g PBmic/kg of consumed NDT which is below the value of 130 g PBmic/kg of consumed NDT that is recommended by the NRC (2001). There was no observed effect for ESPBmic even increasing NDT averages of 52.28, 58.74, 67.32, and 64.38% for levels of 0, 24, 48, and 72% of concentrate in the diet. However, it was observed an effect for the PMIC (g/kgCPB), decreasing linear behavior. In Table 4, it was observed a significant effect in the concentration of CP in cattle, decreasing linear behavior. Also, a significant effect was observed for CC in bovine, increasing linear behavior. Both CP and the DC presented no effects for buffaloes, thus following the same physiological reasoning of cattle. The average CC of buffaloes were higher than those found out for cattle. For CrU was not observed significant effect and the averages were 144.78 mg/dl and 194,91 mg/kg0,75. For the EC, there was no influence of concentrate levels and an overall average of 20,08 g/d.

DISCUSSION

There was no significant effect for VU. Barbosa (2006) studied the effect of urine collection period related to concentrate levels for 25 and 50% of creatinine excretion which could ratify the results of this study. According to the mentioned author, there was no significant influence on the urinary volume in Nelore cattle. While analyzing the results for AL observed in this study, they did not confirm the same behavior that was found out by Leal (2007), who observed an effect of concentrate levels on the AL concentrations when they evaluated daily variations in creatinine excretions and purine derivatives in steers. The percentage that was presented in this study was found out close to the 84%, which was found by Oliveira (2001), thus demonstrating the possibility of obtaining the flow of nitrogen compounds through DP excretion in the urine. In this study, an increase in the consumption of dry, raw MS was observed for cattle with averages of 6.87, 9.91, 12.05, and 13.22, and for buffaloes the values were 7.53, 8.48, 9.73, and 9.56 respectively, for concentrate levels. The results that were presented for the AU may lead us to infer that the observed average was found to be superior to the ones presented fotVerbic (1990), who found 15% concentrations of uric acid. This result is closely linked to the excretion of AL and AU without the addition of xanthine and hypoxanthine.

While analyzing the results of the PABS it is observed that values were higher than those found by Rennó (2000) when working with purine bases, presenting 85.33, 93.59, 102.54, 112.70 and 113.11 averages. It was found levels of 25.0, 37.5, 50.0, 62.5, and 75.0% of concentrate respectively. Leal (2007), when evaluating the daily variations in creatinine excretions and purine derivatives in steers, observed an influence of concentrate levels in relation to PABS. Thus, in the present work, those who consumed more nutrients presented higher concentrations of PABS due to increased microbial synthesis in the rumen. The same behavior was observed for PBmic (g/d). Most likely, an increased dry matter intake allowed the availability of a greater amount of nutrients for rumen fermentation, also allowing greater microbial growth. In addition, the increased rate of passage probably should have occurred whereas increase concentrate intake. The non-influence of ESPBmic in relation to concentrate levels is probably due to protein-energy balance sync from diets. The behavior that was presented for PMIC (g/kgCPB) is probably related to the increased use of MS, and therefore, PB consumption by animals yielded averages of 0.55, 1.12, 1.84, 2.10 for cattle and 0.61, 0.96, 1.49, and 1.48 kg/day for the buffaloes because of the concentration of PB in the experimental diets. When evaluating the results for CP in cattle it may be inferred that the means are found to be within the range of normality for the reference values reported by Gregory et al. (2004), thus indicating that values were 1.35 ± 0.21 mg/dl.

The behavior that was found in cattle for the CC showed that there was an increased glomerular filtration rate when the concentrate levels increased; therefore, the plasma creatinine concentration possibly decreased with decreasing behavior. Since there was no effect of the treatments for CP and CC, it probably influenced that there was not a positive reflex of increased glomerular filtration, the CP concentrations also did not undergo changes in their concentrations, showing that buffaloes have a greater capacity for balance in filtration glomerular in relation to cattle.; the CP concentrations were not modified in their concentrations, thereby showing that buffaloes have a better capacity to balance their glomerular filtration than cattle.

According to Norton (1979), buffaloes have a higher possibility of reabsorption from kidney, which corroborates with the results of this work. As the buffaloes' CC averages were higher than those found by cattle, this may have been due to an increased amount of urine volume that was excreted by buffaloes, although no statistical difference has been observed for the interaction between species, with averages of 8.06, 8.39, 8.56, and 8,20/day for cattle and 21.82, 24.16, 27.34, and 28,95/day for buffaloes. For the CrU, the results showed the same behavior that was found by Leal (2007) when assessing variations in daily creatinine excretion in steers. No statistical difference was found with a mean of 117,92 mg/kg0,75, and despite the fact that average values are below in this work, the behavior was similar. The behavior that was observed for the EC also corroborates the results found by several authors (Valadares et al., 1997; Rennó et al., 2000; 2003; Chizotti, 2006) as they found no significant difference for creatinine excretion. According to Riehl (2004) the endogenous origin of creatinine is determined by its precursor creatine synthesis in the liver and kidneys which participates in the metabolic response of the cells, catabolism in muscles, and it is excreted by the kidneys. The results presented in this study corroborate the justification of the use of creatinine as an effective indicator while using the spot collection method for determination of urine volume, both in cattle and buffaloes, because it is derived from muscle metabolism and is not influenced by the experimental diets.

CONCLUSION

The increase of levels of concentrate in cattle and buffalo diets provides higher rates of purine derivatives as well as the production of microbial protein. Spot urine sampling may be used to estimate the excretion of purine derivatives and also the use of creatine for the estimation of the urinary volume, once that it is not influenced by the experimental diets.

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