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Nitrogenous compounds balance and microbial protein synthesis in steers supplemented with sunflower crushed in partial replacement of soybean meal

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ABSTRACT. Four steers in individual paddocks with Marandu grass (*B. Brizantha*) in 4x4 square design were used to evaluate sunflower crushed supplementation in pasture-grazing animals on nitrogen balance and microbial protein synthesis. Supplements at 6 g kg^{-1} body weight comprised corn, soybean meal, and mineral and soybean meal substituted at proportions 0, 20, 40 and 60%. Diet contained averages 6.79, 6.96, 7.10 and 6.87% nitrogen respectively for substitution levels 0, 20, 40 and 60%. The inclusion of sunflower crushed (SC) increased nitrogen intake and fecal excretion of nitrogen while providing a positive balance. Animals' plasma urea concentration supplemented with SC was 28.13% lower than that of supplemented animals without SC. SC inclusion did not change allantoin concentration, purine derivatives, microbial nitrogen, crude microbial protein and microbial efficiency microbial, with mean rates totaling 150.98 mmol day^{-1}; 158.06 mmol day^{-1}, 112.35 g day^{-1}, 702.18 g day^{-1}; 146.41 crude protein (CP) microbial kg^{-1} of TDN. Partial replacement of soybean meal by sunflower crushed improves nitrogen balance without altering microbial protein synthesis and excretion of urea and creatinine.

Keywords: creatinine, microbial efficiency, plasma urea, purine derivatives, urea.

Balanço de compostos nitrogenados e síntese de proteína microbiana em novilhos suplementados com torta de girassol em substituição parcial ao farelo de soja

RESUMO. Para se avaliar a suplementação de torta de girassol em novilhos mantidos a pasto, sobre o balanço de nitrogênio e a síntese de proteína microbiana, foram utilizados quatro animais em piquetes com capim Marandu (*B. Brizantha*), em quadrado latino 4x4. Os suplementos foram fornecidos na quantidade de 6 g kg^{-1} de peso vivo dia^{-1}; constituídos de milho, farelo de soja e mineral, sendo o farelo de soja substituído nas proporções de 0, 20, 40, e 60%. A dieta disponível apresentava em média 6,79; 6,96; 7,10 e 6,87% de nitrogênio, para os níveis de substituição de 0, 20, 40 e 60%. A inclusão da torta de girassol (TG) elevou o N ingerido e o N fecal, proporcionando balanço positivo. A concentração plasmática de ureia dos animais suplementados com TG foi 28,13% inferior aos animais suplementados sem TG. A inclusão de TG não alterou a concentração de alantoína, os derivados de purina, o nitrogênio microbiano, proteína bruta microbiana e eficiência microbiana, apresentando valores médios de 150,98 mmol dia^{-1}; 158,06 mmol dia^{-1}; 112,35 g dia^{-1}; 702,18 g dia^{-1}; 146,41 proteína bruta microbiana kg^{-1} NDT. A substituição parcial do farelo de soja pela torta de girassol melhorou o balanço de nitrogênio, sem alterar a síntese de proteína microbiana e a excreção de ureia e creatinina.

Palavras-chave: creatinina, eficiência microbiana, ureia plasmática, derivados de purina, ureia.

Introduction

The use of alternative food like sunflower by-products to substitute protein sources may be economically advantageous, especially when soybean meal is directed for other purposes, such as exports (OLIVEIRA et al., 2007). Sunflower crushed is an alternative source of protein and energy with 24 to 33.3 g 100 g^{-1} crude protein, TDN with approximately 79 g 100 g^{-1} and lipid contents with 16.5 g 100 g^{-1} (DOMINGUES et al., 2010; GOES et al., 2010). CP of sunflower crushed is characterized by being widely degradable and has a less than 10 g 100 g^{-1} degradable protein (BERAN et al., 2007). Goes et al. (2008; 2010) found low ruminal degradability of CP in sunflower crushed with 36.65 and 50 g 100 g^{-1} respectively.

Rumen nitrogen may have either an endogenous or a dietary origin. The endogenous nitrogen source is derived from urea recycling, by desquamation of epithelial cells and lysis of the microbial cells. On the other hand, dietary nitrogen is composed of the real protein and non-protein nitrogen (NPN) derived from food intake.

Nitrogen balance is of great importance especially when the use of dietary nitrogen is taken into account. Since it assesses the nitrogen used by ruminal microorganisms, it prevents overfeed protein. The balance of nitrogenous compounds in animals, associated with the concentration of urea in plasma and urine, is a strategy to obtain information on the protein nutrition of ruminants. This fact is important to avoid production, reproduction and environmental losses from the supply of excessive protein or inadequate synchrony energy: protein in the rumen (PESSOA et al., 2009).

The concentration of urea found in urine correlates positively to plasma concentrations of N and N intake and is an indication of the rumen's efficiency. It may also be used as a parameter for protein balance or imbalance: energy diet (VAN SOEST, 1994).

Rumen synthesized microbial protein provides 50% or more of the amino acids available to the animal. It is actually a source of high quality and the different portions of digestible protein fractions escaping ruminal degradation constitute total amino acids that reach the intestine. Microbial protein synthesis depends on such factors as the source of nitrogen and carbohydrates in the diet, ruminal dilution rate, frequency of feeding, consumption of food, forage: concentrate ratio, ionophores and minerals, such as P, S and Mg, in the diet (PEREIRA et al., 2001; PEREIRA et al., 2007).

Ingested amino acid may be fermented by microorganisms as a source of energy or it may be incorporated into microbial protein. Since microbial growth is dependent on the supply of fermentable carbohydrates, the products of protein metabolism is influenced by the availability of carbohydrates. When ATP originates from the fermentation of carbohydrates, the amino acid is available and may be incorporated into microbial protein. If ATP is not sufficient to allow protein synthesis, amino acids are fermented for energy and an accumulation of ammonia occurrs. If ammonia production in the rumen is greater than its rate of microbial incorporation, it will be absorbed with an increased activity of urea recycling in the liver and kidneys, necessary to protect the animal from its toxic effect.

Current research determines the balance of nitrogen and microbial protein synthesis in steers fed on pasture and supplemented with sunflower crushed as a replacement of soybean meal.

Material and methods

The experiment was conducted at the Federal University of Grande Dourados (UFGD), in Dourados MS Brazil, between October and November 2009 (Table 1) for 52 days.

Table 1. Maximum (Tmax) and minimum (Tmin) temperature, maximum (URmax) and minimum (URmin) relative humidity and rainfall (Prec) in Dourados MS Brazil, during October and November, 2009.

Month	Tmax (°C)	Tmin (°C)	URmax (%)	Urmin (%)	Prec (mm)
October	29.76	18.64	93.61	33.75	11.59
November	33.40	21.17	92.50	47.00	5.00

UFGD - 2009 Meteorological data.

Four crossbred steers, approximately 18 months old and average weight 285 kg were used. They were fitted with a permanent ruminal cannula, dewormed with Ivermectin (1%), kept in individual paddocks of Marandu grass (*B. brizantha*), in a 4 x 4 Latin square design.

Each experimental period lasted 13 days including 10 days for adaptation. Concentrate was supplied daily in a trough at 6 g kg^{-1} of body weight, in the morning until 10:00, so that forage intake would not be disturbed. At the end of each experimental period, the animals were weighed and the supplements adjusted according to the weight obtained.

Treatments were balanced to contain 28% CP and were composed of sunflower crushed crushed as a partial replacement for soybean meal at 0, 20, 40 and 60% (Table 2). Table 3 shows the chemical compositions of the ingredients.

The experimental area comprised two hectares, divided into four paddocks, separated by an electric fence, with drinking and feeding troughs. *B. brizantha* cv Marandu pasture was planted in 2008 through an integrated crop and livestock system, after corn culture.

Table 2. Ingredients (g kg^{-1} as feed) and chemical composition of concentrates (g 100 g^{-1} Dry Matter - DM).

Ingredients	C0#	C20#	C40#	C60#
Corn	426	357	287	218
Soybean meal	524	419	315	210
Sunflower crushed	--	174	348	522
Mineral	50	50	50	50
Parameters	Chemical composition (g 100 g^{-1} of DM)			
DM	91.51	87.46	87.97	91.03
CP	29.35	27.87	27.91	27.46
EE	3.68	5.65	8.82	11.00
NDF	26.98	29.32	30.44	32.48
ADF	5.51	13.86	17.16	18.73
TDN⋆	85.12	82.00	78.38	76.00
MM	3.25	3.19	4.30	5.28

⋆% NDT = 9.6134+0.829DMS (r^2 = 0.98). Capelle et al. (2001). DMS = *in vitro* dry matter digestibility. # C00 = Concentrate without sunflower crushed; C20 = concentrate with 20% of soybean meal replaced by sunflower crushed; C40 = concentrate with 40% of soybean meal replaced by sunflower crushed ; C60 = concentrate with 60% of soybean meal replaced by sunflower crushed. DM = dry matter, CP = crude protein, EE = ether extract

Table 3. Chemical composition of ingredients used in concentrate for steers, and Marandu grass consumed.

Ingredients	DM*	CP*	EE*	NDF*	ADF*	MM*	IVDDM*
Soybean Meal	85.64	50.99	6.71	34.14	20.08	9.68	95.40
Corn	87.86	11.68	3.28	13.93	5.43	1.70	98.80
Sunflower crushed	95.05	30.93	16.76	42.69	31.27	4.72	64.54
Mineral	96.31	-	-	-	-	-	-
Marandu grass	15.88	15.22	5.17	81.98	36.90	2.87	79.10

DM = dry matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, MM = mineral matter, IVDMM = *in vitro* dry matter digestibility. * % dry matter

On the first day of the experiment, total dry matter availability was determined by a cut close to the ground of 10 randomly delimited areas, by metal squares (0.25 m^2) within the same paddock. The collection of forage intake by animals occurred on the 13th day of each experimental period by the emptying of the rumen after 12 hours of fasting. All the samples were stocked in plastic bags, labeled and transported to the Laboratory of Animal Nutrition / FCA / UFGD.

Determination of dry matter intake was based on the relationship between an external (chromium oxide, Cr_2O_3) and an internal (iADF) marker. Further, 10 g of Cr_2O_3 were introduced by a rumen cannula into the animals' rumen, from the second day of the experiment, at 08:00 and 17:00, for a 10-day period, with five days adaptation and five days collection (SOARES et al., 2003).

Feces samples were collected directly from the rectum of the animals at the same time as chromium oxide was supplied. Samples were packed in properly identified plastic bags and frozen at -10°C. At the end of each period, a sample from each animal was retrieved, in each paddock and for each period. Chromium in the feces was analyzed by atomic absorption spectrophotometry, following Willians et al. (1962).

The following formula was used to determine fecal dry matter production: g DM feces excreted per day = (100 x Cr_2O_3 supplied) / (% of Cr_2O_3 in fecal DM). Indigestible ADF was used to estimate forage intake, following procedure by Penning and Johnson (1983) and adapted by Detmann et al. (2003), based on in situ degradability, for 144 hours.

The dry matter intake was determined by the equation DMI = {[(EF x CIFZ) - IS] / CIFO} + CMSS, where, DMI = dry matter intake (kg day^{-1}); EF = fecal excretion (kg day^{-1}); CIFZ = concentration of index in the feces (kg kg^{-1}), IS = index in the supplement (kg day^{-1}); CIFO = concentration of index in the forage (kg kg^{-1}), CMSS = consumption of supplement's dry matter (kg day^{-1}).

The urine collection was performed on the 12th day of the experiment, spot mode¸ four hours after the supply of the supplement from the animals' spontaneous urination. Urine was stored in two aliquots, or rather, the first aliquot 15 ml of urine and 135 ml sulfuric acid 0.036 N was used to determine the concentration of urinary creatinine, urea, uric acid and allantoin. The second aliquot with 100 ml urine and 1 ml sulfuric acid 36 N was used to determine total N concentration in urine. The samples were immediately frozen at -20°C for later analysis.

Allantoin was determined by the calorimetry method, according to technique by Chen and Gomes (1992). Commercial kits (Labtest® and Gold Analisa®) were used to determine creatinine and uric acid concentration. Total excretion of purine derivatives (PD) was calculated by the sum of allantoin and uric acid excreted in the urine, expressed in mmol day^{-1}. Microbial purine absorbed (Pabs, mmol day^{-1}) was calculated from the excretion of purine derivatives (PD mmol day^{-1}) by the equation proposed by Verbic et al. (1990): DP = 0.85 Pabs + 0.385$PV^{0.75}$, where 0.85 = retrieval of absorbed purine as urinary derivatives of purine; 0.385 $PV^{0.75}$ = endogenous contribution for purine excretion.

The synthesis of rumen microbial nitrogen (g N mic day^{-1}) was determined in terms of microbial purines absorbed (Pabs, mmol day^{-1}) with the equation: Nmic = (70 x Pabs)/0.83 x 0.116 x 1000, where 70 = N content of purines (mg N mol^{-1}); 0.116 = ratio N purine: N total in bacteria, 0.83 = digestibility of microbial purines.

Urine volume was calculated as follows: VU (L / d) = (27.36 x PV) / [creatinine], where 27.36 is the average daily excretion of creatinine in ppm, obtained by Rennó et al. (2000) for zebu cross steers; PV is the live weight of the animal and [creatinine] is the concentration of creatinine in mg L^{-1}, found in the animals' spot sample. The daily excretion of N-urea and N-creatinine was obtained by the product of urea and creatinine concentrations in a 24-hour urine volume, multiplied by 0.466 and 0.3715, corresponding to N in urea and creatinine, respectively.

Samples of feces and urine were evaluated for nitrogen content, according to AOAC methodology, described by Silva and Queiroz (2002). A composed sample per animal was performed at the end of

period. Nitrogen balance (NB) was calculated as the difference between intake of total nitrogen and urine and feces excretion. The latter rates quantified nitrogen retention (nRet), discounting the estimated value of NB requirement for basal endogenous nitrogen (NEB = $0.35PV^{0.75}$).

At 7:00 am on days 0, 3, 6, 9 and 12, blood samples were taken by jugular vein puncture. Heparinized Vacutainer® tubes, centrifuged at 3000 rpm for 15 minutes to remove the plasma, were employed. The resulting plasma was stored in micro tubes and frozen at -20ºC for the analysis of plasma urea levels. After thawing, the plasma urea was determined by calorimetry furnished by a commercial kit (Gold Analisa®).

Statistical analyzes were performed by the experiment's principal design of 4x4 Latin square. Analyses of regression were performed with Statistical Package SAEG 9.1 (UFV, 2007).

Results and discussion

During the experiment, total available dry matter was 3666.11 kg DM ha^{-1} and green dry matter availability was 2999.52 kg ha^{-1} (Table 4). These rates were close to those obtained by Silva et al. (2009) who pointed out that total dry matter and green dry matter should be 4500 kg DM ha^{-1} and 1200 kg ha^{-1} for animal selectivity. There was no effect for dry matter intake by steers with an average rate of 6.59 kg day^{-1} (Table 4), although effect occurred for nitrogen intake (Table 5).

The nitrogen (N) diet had an average of 6.79; 6.96; 7.10 and 6.87 g 100 g^{-1} respectively at levels 00, 20, 40 and 60%. The inclusion of sunflower crushed linearly increased the nitrogen intake by 24.18% (Table 4). This may be due to the protein composition of diet linked to the animals' dry matter intake. Although total dry matter intake is not affected by the inclusion of sunflower crushed, the substitution levels 20, 40 and 60 were 15.57%; 14.42; 2.29% above the supplement without the addition of sunflower crushed. In fact, the latter may have caused the increased consumption of CP or N. Pereira et al. (2011b), who provided sunflower crushed for cows in the proportion of 0, 7, 14 and 21% in the concentrate, did not report any inclusion effect of the co-product in nutrient intake. Average consumption for crude protein was 1.8 kg day^{-1} or 288 g N day^{-1}, very close to rates in current assay for 20 and 40% substitution levels.

The inclusion of sunflower crushed increased N fecal excretion without changing N urinary N, which provided positive nitrogen balance. Further, 20 and 40% levels were the highest values for fecal N and nitrogen balance, with lower urinary losses. The effect of increasing N excretion in feces for treatments with inclusion of sunflower crushed may be related to higher concentrate intake and consequently to N. Although supplement intake did not show any significant effect, a slight increase occurred. This was due to the fact that nitrogen balance was positive, indicating that protein was retained in the animal body, with conditions for weight gain in the experimental animals.

A positive correlationship occurs between concentrations of plasma and urinary urea and between concentration of urea in urine with N intake and protein:energy relationship (HARMEYER; MARTENS, 1980; VAN SOEST, 1994). In current assay, highest N intake did not cause high urinary N excretion, perhaps due to diet quality that showed no protein deficiency when compared to energy.

Table 4. Dry matter intake of forage (DMF), supplement (DMSS) and total (DMT) in kg day^{-1}.

	Substitution levels (%)							
	0	20	40	60	Average	CV(%)	SEM*	P < F
DMF (kg day^{-1})	4.53	5.23	4.93	4.16	4.71	44.58	1.93	*****
DMSS (kg day^{-1})	1.56	1.81	2.05	2.09	1.88	20.77	0.41	0.043
DMT (kg day^{-1})	6.10	7.05	6.98	6.24	6.59	28.07	1.71	*****

DMF: Y = 4.17; DMSS: Y = 0.0092x+1.603 (r^2 = 0.92); DMT: Y = 6.59. *Mean standard error.

Table 5. Averages of nitrogen (N) intake, fecal N, urinary N, N excretion, nitrogen balance (NB), basal endogenous nitrogen (NEB) and retained nitrogen (N Ret), in g day^{-1} in steers supplemented with sunflower crushed as partial replacement of soybean meal.

	Substitution levels (%)							
Parameters	0	20	40	60	Average	CV (%)	SEM	P<F
N intake	168.75	211.83	221.82	195.04	199.36	4.51	27.49	0.0056
N fecal	19.48	25.71	23.86	22.21	22.81	7.77	3.04	0.0101
N urinary	41.29	29.92	26.48	41.29	34.74	37.73	14.26	0.1904
N excretion	60.76	55.63	50.34	63.49	57.55	24.77	14.56	******
NB	107.98	156.20	171.48	131.56	141.80	12.60	34.13	0.0075
NEB	24.28	25.54	22.99	24.21	24.26	-	0.80	-
N Ret	83.70	130.66	148.49	107.35	117.55	12.00	34,29	0.0075

N intake: Y = 0.003x+3,41 (r^2 = 0.99); N fecal: Y = 0.005x^2+0,32x+19.89 (r^2 = 0.83); N urinary: Y = 0.016x^2-0.99x+41,81 (r^2 = 0,97); N excretion: Y = 57.55; NB: Y =-0.055x^2+3,73x+106.87 (r^2 = 0.98); NRet: Y = -0.055x^2+3,75+82.81 (r^2 = 0.98). *Mean standard error.

The excretion of urea and N-urea was constant in all treatments with averages 162.5 mgU kg^{-1} BW for urea and 85.04 for N-urea (Table 6). Rennó et al. (2000) registered rates of 184.85 mgU kg^{-1} BW and 86.14 mg dL^{-1} when protein levels were close to 12%. The daily excretion of creatinine and N-creatinine did not change significantly. Current assay reported average rates of 22.59 mgC kg^{-1} BW and 8.46 mg dL^{-1} respectively. Chizzotti et al. (2006) reported no effect on creatinine excretion in calves, which remained constant in different diets.

Ørskov and MacLeod (1982) suggested that the creatinine excretion could predict N endogenous excretion (NUE) which amounted to 7.42 g N day^{-1} for animals with an average weight of 285 kg. This rate is close to creatinine excretion in animals without sunflower crushed supplements (7.71 gN day^{-1}). Creatinine is a metabolic product that the body does not need any more. Since it is not used for the formation of new molecules, it is excreted by the kidneys (LEAL et al., 2007). The daily production of creatinine (and, consequently, creatinine excretion) depends on muscle mass and is proportional to the animal's weight. According to the NRC (2000), if an animal is fed on a diet with adequate energy amounts, protein percentage decreases and fat percentage increases in the empty body as its weight approaches maturity status. The percentage of muscle tissue in growing animals varies according to animal weight and, consequently, the creatinine excretion may be changed. Adult animals vary less in body composition and therefore creatinine excretion as a function of live weight becomes less variable (LEAL et al., 2007). Since animals in current study were in the growth phase, creatinine excretion might have been affected.

The plasma concentration of urea in animals supplemented with sunflower crushed presented a quadratic behavior and an average of 19.49 mg dL^{-1}, which is 28.13% lower than in supplemented animals without sunflower crushed, with mean rates of 27.12 mg dL^{-1}, close to those registered by Rennó et al. (2008) for zebu. Domingues et al. (2010) reported lower peaks for supplemented animals with sunflower crushed replacing cottonseed meal with a peak two hours after feeding. In current assay, highest peaks occurred between 2-4 hours after feeding. Urea is synthesized in the liver from N-NH_3 by protein catabolism and by absorption through the rumen wall. It is then metabolized and transformed into urea. The process requires energy expenditure by the animal so that urea N-NH_3 could be metabolized to avoid toxicity.

Broderick et al. (1993) proposed that less than 11 mg dL^{-1} concentrations of plasma urea in beef cattle indicated PDR deficiency in rations. Probably this did not occur in current assay, since rates were higher than those reported by the former authors. The nutritional quality of the diet is thus confirmed since there was no CP deficiency when compared to NDT. Rate for plasma urea was 21.39 mg dL^{-1}, or rather, below the limits beyond which diet N losses would be occurring. According to Oliveira et al. (2001), rate is over 24 to 25 mg dL^{-1} blood.

Albeit not significant, the fractional excretion of urea showed an increasing behavior as a function of increasing levels of concentrate feed. Valadares et al. (1997) concluded that the fractional excretion of urea is variable with greater retention of urea at low intakes and increased excretion at high N intakes.

The replacement of soybean meal by sunflower crushed did not alter concentration of allantoin, purine derivatives, absorbed purine, microbial nitrogen, microbial crude protein (CPmic) and microbial efficiency (Emic) of animals, respectively averaging 150.98 mmol day^{-1}, 158.06 mmol day^{-1}; 154.54 mmol day^{-1}, 112.35 g day^{-1}, 702.18 g day^{-1}; 146.41 gCPmic kg^{-1} of NDT (Table 7).

Table 6. Averages for urea concentration in urine, urea excretion, creatinine concentration in urine creatinine excretion, plasma urea and creatinine, fractional excretion of urea, N-urea (ENUrea) and N-creatinine (ENCreatinine).

	Substitution levels (%)							
Parameters	0	20	40	60	Average	CV (%)	SEM	P<F
Urea in urine (mgU dL^{-1})	3.36	3.64	3.36	3.67	3.51	44.93	1.42	*****
Urea excretion (mgU kg^{-1} BW)	118.28	201.89	143.95	186.10	162.56	55.89	88.20	*****
Creatinine urine (mg dL^{-1})	72.41	47.49	52.77	29.05	50.43	51.03	28.01	0.0414
Creatinine excretion (mgC kg^{-1} BW)	21.88	25.14	26.22	17.12	22.59	66.68	13.96	*****
Plasma urea (mg L^{-1})	27.12	18.51	20.06	19.91	21.39	18.57	4.97	0.0318
Plasma Creatinine (mg dL^{-1})	7.43	4.66	5.44	5.93	5.87	8.46	1.13	0.0005
Fractional excretion of urea (%)	1.92	2.24	2.30	6.82	3.32	97.17	3.56	0.0811
ENUrea (mgN-U kg^{-1} BW)	92.30	94.08	67.08	86.72	85.05	55.89	4.11	*****
ENCreatinine (mgN-c kg^{-1} BW)	8.20	9.43	9.83	6.41	8.48	66.68	5.24	*****

Urea in urine: Y = 3.51; Urea excretion: Y = 162.56; Creatinine urine: Y = -0.62x+69.15 (r^2 = 0.81); Creatinine excretion: Y = 22.59; Plasma urea: Y = 0.005x^2 -0.417x+26.52 (r^2 = 0.84); Plasma Creatinine: Y = 0.002x^2 - 0.1409x + 7.24 (r^2 = 0.82); Fractional excretion of urea: Y = 0.0026x^2 - 0.0837x + 2.156 (r^2 = 0.93); ENUrea: Y = 55.89; ENCreatinine: Y = 66.68. *Mean standard error.

Table 7. Averages of urinary volume (VU), allantoin (ALA), uric acid (UA), purine derivatives (PD), absorbed purine (Pabs), microbial nitrogen (N mic), microbial crude protein (CPmic) and microbial efficiency (Emic) of steers supplemented with sunflower crushed in partial replacement of soybean meal.

	Substitution levels (%)							
Parameters	0	20	40	60	Average	CV (%)	SEM	P<F
VU (L day^{-1})	9.39	14.35	13.57	14.44	12.93	35.37	3.91	0.1018
ALA (mmol day^{-1})	108.19	138.97	153.08	203.71	150.98	61.81	77.48	0.0818
AU (mmol day^{-1})	4.99	6.81	6.97	9.54	7.07	19.70	2.14	0.0009
DP (mmol day^{-1})	113.18	145.77	160.05	213.25	158.06	59.51	78.79	0.0724
Nmic (g day^{-1})	73.96	101.84	114.05	159.55	112.35	71.61	67.39	0.0724
Pabs	101.74	140.08	156.88	219.46	154.54	58.56	92.69	0.0724
CPmic (g day^{-1})	462.25	636.48	712.80	997.21	702.18	71.66	42.12	0.0724
Emic (gCPmic $kgTDN^{-1}$)	69.82	130.73	147.73	210.38	146.41	72.70	90.86	0.0769

VU: Y= 0.0718x+10.78 (r^2 = 0.60); ALA: Y = 1.50x+105.89 (r^2 = 0.94); AU: Y =-0.069x+5.006 (r^2 = 0.91); DP: Y = 1.57x+110.86 (r^2 = 0.91); Nmic: Y = 1.134x+72.00 (r^2 = 0.94); Pabs: Y = 1.85x+99.04 (r^2 = 0.94); CPmic: Y = 8.40x+450.01 (r^2 = 0.94); Emic: Y = 1.788x+92.76 (r^2 = 0.94);. *Mean standard error.

Microbial protein synthesis depends on the availability of carbohydrates and nitrogen in the rumen (NRC, 2001; MAGALHÃES et al., 2005). Synchronization between the availability of fermentable energy and nitrogen degradable should exist to maximize microbial growth. The efficiency of microbial growth depends on energy partition in the maintenance and growth. It is inversely proportional to the microorganisms' permanence in the rumen. The faster the passage of microorganisms, the lower is the energy required for maintenance. No protein deficit occurred in current assay with regard to energy. TDN : CP ratio was 5.04. Rate of 146.41 gCPmic $kgTDN^{-1}$ is similar to that found by Pereira et al. (2011a) who studied the inclusion of sunflower crushed in cows. Rate is very close to 130 g kg^{-1} of NDT recommended by NRC (2001).

Allantoin rates were lower than those reported by Pereira et al. (2011a) who included 0, 7, 14 and 21% of sunflower crushed in diets for lactating cows. Highest inclusion of sunflower crushed maximized animals' creatinine excretion, with 241.0 mmol day^{-1}. Rennó et al. (2000) and Magalhães et al. (2005) evaluated increasing levels of urea in steers and obtained averages 112 and 167.9 mmol allantoin day^{-1}. Data variability in the literature stems from several factors, with special reference to roughage and diet concentrate proportions, fiber percentage and degradable protein percentage in the rumen (CASTAÑEDA et al., 2009).

Concentrations of uric acid were influenced by inclusion of sunflower crushed with a 52.30% increase for the highest substitution level. In spite of the above increase, rates were lower than those found in the literature (CASTAÑEDA, et al., 2009; OLIVEIRA et al., 2001). Chen and Gomes (1992) maintain that the proportion of uric acid in purine derivatives (DP) ranges between 15 and 20% and is very constant in the same animal, although varies between animals. However, in current assay, proportions had a mean of 4.40 and 4.67, 4.35 and 4.47%, or rather, constancy is demonstrated. Castañeda et al. (2009) obtained rates between 6.4 and 10.4% and Chizzotti et al. (2006) reported an average rate of 8.25% of uric acid in DP.

Conclusion

The partial replacement of soybean meal by sunflower crushed up to 60% improved nitrogen balance and reduced plasma urea in animals kept on Marandu pasture, with better utilization of nitrogen intake. Supplementation with sunflower crushed does not alter urea excretion, urinary creatinine levels, urinary allantoin and purine derivatives.

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