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Coffee hull in the diet of dairy heifers: nitrogen balance and microbial protein synthesis[1]

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ABSTRACT - It was evaluated nitrogen compounds and microbial protein synthesis in heifers fed diets containing coffee hulls (0.0; 8.75; 17.25; and 26.25% of dry matter) replacing ground corn concentrate at the following levels of coffee hulls in the total diet dry matter: 0.0, 3.5, 7.0 or 10.5%. It was used 24 crossbreed heifers (7/8, 15/16 and 31/32 Holstein-Zebu), which were distributed in a random block design made up accordingly to the weight of the animals. Spot samples of urine were colleted aproximatelly four hours after morning feeding and were used to estimate microbial protein synthesis by using urine purine derivatives. It was not observed effect of coffee hull levels in the diet on total nitrogen intake (160 g/day) and nitrogen excretion in the urine (87.4 g/day). The inclusion of coffee hull in the diet linearly increased nitrogen excretion in feces, as well as nitrogen balance. There was linear reduction in urinary excretion of allantoin, in total purine derivative and absorbed purine, which reduced 0.715, 0.873, and 0.954 mmol/day to each coffee hull unity added to the concentrate, respectively. Coffee hull altered microbial protein synthesis, which reduced in 0.687 g/day to each coffee hull unity added to the concentrate. Reduction in microbial protein synthesis can reduce weight gain in heifers fed coffee hulls.

Key Words: agroindustrial residue, creatinine, N-urea, purine derivatives, spot urine

Casca de café em dietas para novilhas: balanço de compostos nitrogenados e síntese de proteína microbiana

RESUMO - Avaliaram-se o balanço de compostos nitrogenados e a síntese de proteína microbiana (PBmic) em novilhas leiteiras submetidas a dietas contendo casca de café (0,0; 8,75; 17,5 e 26,25% da matéria seca) em substituição ao milho no concentrado, correspondendo aos níveis de 0,0; 3,5; 7,0 e 10,5% da matéria seca da dieta total. Foram utilizadas 24 novilhas mestiças com grau de sangue Holandês variando de 7/8 a 31/32, distribuídas em delineamento em blocos casualizados, formados de acordo com o peso dos animais. Amostras *spot* de urina foram coletadas aproximadamente 4 horas após a alimentação da manhã e utilizadas para estimar a síntese de PBmic utilizando-se os derivados de purina na urina. Não foi observado efeito dos níveis de casca de café na dieta sobre o consumo de nitrogênio total (160 g/dia) e a excreção de nitrogênio na urina (87,4 g/dia). A inclusão de casca de café na dieta promoveu aumento linear na excreção de nitrogênio nas fezes, bem como alteração do balanço de nitrogênio. Houve redução linear nas excreções urinárias de alantoína, nos derivados de purinas totais e purinas absorvidas, que reduziram 0,715; 0,873; 0,954 mmol/dia a cada unidade de casca de café adicionada no concentrado, respectivamente. A casca de café alterou a síntese de PBmic, que teve redução de 0,687 g/dia a cada unidade de casca de café adicionada ao concentrado. A redução na síntese de proteína microbiana pode diminuir o ganho de peso em novilhas alimentadas com casca de café.

Palavras-chave: creatinina, derivados de purina, N-ureia, resíduo agroindustrial

Introduction

Heifer production is an important component of the dairy cattle production system. The continuity of the productive process, genetic progress of the herd, improvements in the conformation characteristics and the introduction of more fitted animals to the environmental conditions of the farm are directly influenced by this animal category.

An efficient heifer raising system should give the animals conditions to reach puberty and present the first birth as early as possible, with adequate weight

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depending on each breed. According to the NRC (2001), young animals need less net energy and metabolizable protein for weight gain and maintenance, consequently they are more efficient than older and heavier animals.

According to Costa et al. (2007), heifer production can be 15 to 20% of the total cost of milk production, and 50% of this is related to animal feeding. In this context, the use of alternative feedstuffs to the roughage and concentrates traditionally used in the diets of these animals becomes important, and its technical and economic feasibility would depend on their nutritive value and relative cost, therefore research studies to assess their potential for use are required.

Among the many residues produced by agroindustry, coffee hull is available in several states in Brazil, so its assessment is necessary for potential use in the heifer diet (Souza et al., 2006a) and dairy cattle diet (Souza et al., 2005; Souza et al., 2006b), to replace both concentrated feedstuffs (Souza et al., 2005; Souza et al., 2006a; Souza et al., 2006b) and roughage (Rocha et al., 2006; Teixeira et al., 2007).

According to Souza et al. (2001, 2005), more than 30% of the coffee hull nitrogen adheres to the fibrous fraction reducing nitrogen availability for microorganisms in the rumen. Hence, the assessment of the nitrogen balance and the efficiency of the microbial protein synthesis in animals submitted to different diet conditions becomes a valuable tool for nutritional assessment of feedstuffs.

Therefore, an experiment was carried out to assess the balance of nitrogen compounds and microbial protein synthesis in heifers submitted to diets containing different levels of coffee hull.

Material and Methods

The experiment was carried out in the Dairy Cattle Teaching, Research and Extension Unit at the Departamento de Zootecnia at the Universidade Federal de Viçosa from February 14th to May 18th, 2001. It was used twenty-four 7/8, 15/16, pure and crossbred Holstein Zebu heifers at approximately 12 months of age and initial average weight of 200 kg. The animals were placed in a randomized block design with six replications and the blocks were formed according to the weight and blood degree of the animals.

The diets (Table 1) consisted of four coffee hull levels (0, 8.75, 17.5 and 26.25% on the concentrate dry matter base), replacing the ground corn of the concentrate, corresponding to the levels of 0, 3.5, 7.0 and 10.5% coffee hull in the total dry matter of the diet. The diets (isoprotein with approximately 15.5% crude protein) consisted of 60% haylage Tifton-85 grass and 40% concentrate and were supplied *ad libitum* twice a day, at 7 a.m. and 5 p.m., to allow daily leftovers of 10% of the quantity offered.

The heifers were housed in individual 8 m^2 stalls with a cement floor, equipped with a feeder, drinkers and a bed area for the animals with sawdust. At the start of the adaptation period, the animals received 1.0 mL/50 kg body weight ADE vitamin complex and were treated against endo and ectoparasites.

The experimental period consisted of 10 days of adaption and 84 days of data collection, subdivided into three 28-day periods, when the food and leftover samples were collected and the animals were weighed. The feces samples were obtained directly in the animal rectum and collections were

Table 1 - Nutritional composition and percentage of the ingredients in the experimental diet

Item	Tifton-85 haylage	Coffee hulls	Concentrate (Coffee hulls, % of DM)			
			0	8.75	17.5	26.25
Dry matter %	46.8	87.3	88.4	88.4	88.3	88.1
Organic matter (%DM)	91.4	92.9	93.6	92.9	92.3	91.7
Crude protein (%DM)	16.5	8.6	13.7	13.7	13.5	14.1
Acid detergent insoluble nitrogen (% total nitrogen)	3.0	22.9	2.3	4.7	6.9	9.2
Ether extract (%DM)	1.6	0.8	4.3	3.9	3.6	3.0
Neutral fiber detergent without ash and protein (%DM)	62.8	53.4	9.7	13.1	16.7	20.1
Indigestible acid detergent fiber (%DM)	9.4	35.8	0.8	3.6	6.4	10.9
Lignin (%DM)	4.1	14.6	0.6	2.0	3.5	5.0
Total digestible nutrients[1]	57.1	44.9	84.3	79.7	75.5	71.0
Ingredient (% DM)						
Tifton-85 haylage			60.0	60.0	60.0	60.0
Soybean meal			7.82	7.78	7.68	7.68
Ground corn			30.36	26.9	23.5	20.0
Coffee hulls			0.0	3.5	7.0	10.5
Mineral mix			1.82	1.82	1.82	1.82

[1]Values in percentage of the DM.

made at 8 a.m., 12 p.m., and 4 p.m. on the 16th, 18th and 20th days, respectively, of the third experimental period.

The fecal excretion was estimated by using the indigestible acid detergent fiber (IADF) as internal indicator, which was obtained from the feedstuff, leftover and feces samples according to Cochran et al. (1986), with some alterations, because they were placed in *Ankon* (*Filter bag* F57) bags and incubated in the rumen of a lactating cow for a period of 144 hours. The remaining material from the incubation was washed with water and then submitted to extraction with acid detergent solution, whose residue was considered indigestible fiber in acid detergent.

The urine volume of each animal, used to calculate the daily total nitrogen excretion, urea and purine excretion (allantoin and uric acid) was quantified by multiplying the value of the daily creatinine average excretion (27.76 mg/kg body weight, obtained by Rennó et al. (2008), by the mean weight of the animals in the third experimental period, where the mean values of 279.2, 267.9, 269.8 and 254.3 kg for the levels of 0.0, 8.75, 17.5 and 26.25% coffee hull, respectively, were observed. This product was then divided by the creatinine concentration, in mg/L, detected in the spot urine samples of the respective animals. The spot urine samples of all the animals were collected on the eighth day of the third experimental period, approximately 4 hours after the morning treatment. An aliquot 10 mL urine of each sample was diluted in 40 mL H_2SO_4 and 0.036 N to reduce the pH to levels below 3, thus preventing nitrogen losses and bacterial destruction of the purine. The samples were then placed in plastic pots and stored in a freezer at -4°C for later analyses of urea, creatinine, allantoin and uric acid.

The allantoin was analyzed by the colorimetric method, as described by Chen & Gomes (1992). The urea, creatinine and uric acid were analyzed by using commercial kits from the Labtest Company. The absorbed microbial purine (AP, mmol/day) was calculated from the purine excretions ($\hat{Y}$, mmol/day), by using the equation $\hat{Y} = 0{,}85\ AP + 0{,}385\ BW^{0,75}$, where 0.85 was the recovery of the purine absorbed as urine purine derivatives and $0.385\ BW^{0,75}$ was equivalent to the endogenous purine excretion (Verbic et al., 1990). The total purine derivative excretion was estimated from the sum of the quantity of uric acid and allantoin excreted in the urine that, when added, represented approximately 98% of the daily excretions of the purine derivatives in dairy cattle (Rennó et al., 2000).

Microbial protein production (PBmic, g/day) was calculated from the absorbed purine (AP mmol/day) using the equation described by Chen and Gomes (1992), in which the value of the N-purine:N-total ratio in the bacteria was changed from 0.116 to 0.117, as reported by Rennó et al. (2000): Nmic = (70 PA) / (0.83 x 0.117 x 1000), where 70 is the purine nitrogen content (mgN/mmol) and 0.83 the microbial protein digestibility.

The contents of organic matter, total nitrogen, fiber in neutral detergent, ether extract, lignin and insoluble nitrogen in acid detergent (Table 1) were determined in the foodstuffs, leftovers and feces samples following procedures reported by Silva & Queiroz (2002).

The data was submitted to analysis of variance and regression at the level of 5% significance, using the SAEG program, version 7.1 (UFV, 1997). The best model was chosen based on the coefficient of determination and the significance of the regression coefficients by using the Student t test at 5% probability.

Results and Discussion

The daily averge intake of coffee hull dry matter was, respectively, 0, 228, 465 and 706 g for the animals fed on concentrated diets containing 0, 8.75, 17.5 and 26.25% of this residue, corresponding to the levels of 0, 3.5, 7.0 and 10.5% coffee hull in the dry matter of the diet (Souza et al., 2006a).

No effect was observed (P>0.05) for coffee hull on nitrogen intake, nitrogen excretion in the urine expressed in g/day and nitrogen excretion in the form of urea in the urine and urea in the urine expressed in mg/kg live animal weight (Table 2), and mean values recorded were 160 and 87.4 g/day and 290.5 and 691.2 mg/kg live weight, respectively. In studies with crossbred and pure Holstein dairy heifers, in which coffee hull replaced corn silage up to 21% of the total dry matter of the diet, Teixeira et al. (2007) reported increase (P<0.05) in nitrogen intake and excretion in the urine and recorded values of 0.51 and 0.26 g at each percentage unit of coffee hull added. According to Van Soest (1994), nitrogen excretion in the urine is greater when the crude protein concentration in the diet and nitrogen ingestion by the animal are increased.

Linear reduction (P<0.05) was observed for nitrogen in the form of urea in the urine and urea in the urine expressed in g/day, and a reduction of 0.856 and 1.83 g was estimated for each percentage unit of coffee hull added to the concentrated feed, respectively. The high nitrogen concentrations in the form of insoluble nitrogen in neutral detergent and insoluble nitrogen in acid detergent present in the coffee hull may have reduced the quantity of protein degraded in the rumen, reflecting a smaller ammonia production. The smaller availability of rumen ammonia may have contributed to the smaller excretion of nitrogen in the form of urea observed for the animals submitted to the diets containing high levels of coffee hull. These results were reinforced by the fact that the nitrogen excretion in the feces

Table 2 - Means of nitrogen intake and excretion in the feces and urine expressed in different forms in function of the level of coffee hull in the concentrated feed

Item	Diet				CV (%)	Regression equation
	0,0	8.75	17.5	26.25		
Nitrogen intake, g/day	163.0	160,3	162.2	153.5	15.5	$\hat{Y} = 160.0$
Fecal nitrogen, g/day	47.5	51.4	60.3	57.9	19.3	$\hat{Y} = 48.27 + 0.4572\,X$ (r2 = 0.78)
Urinary nitrogen, g/day	99.0	88.1	75.6	85.7	21.4	$\hat{Y} = 87.4$
Urine urea nitrogen, mg/kg LW	322.1	311.7	251.6	276.8	18.5	$\hat{Y} = 290.5$
Urine urea nitrogen, g/day	90.9	83.7	67.8	71.2	25.1	$\hat{Y} = 89.66 - 0.8563\,X$ (r2 = 0.81)
Urinary urea, mg/kg LW	691.2	668.8	540.0	593.4	18.5	$\hat{Y} = 691.2$
Urinary urea, g/day	195.1	179.6	145.5	152.9	25.1	$\hat{Y} = 192.41 - 1.8376\,X$ (r2 = 0.81)
Nitrogen balance, g/day	+16.5	+20.8	+26.3	+9.9	65.6	$\hat{Y} = 15.38 + 1.6070\,X - 0.0674\,X^2$ (r^2 = 0.81)

CV= coefficient of variation

was linearly influenced (P<0.05) and an increase of 0.457 g/ percentage unit of coffee hull added to the concentrated feed was estimated.

Teixeira et al. (2007) also observed an increase in fecal nitrogen excretion of 0.45 g/coffee hull unit added in an experiment with coffee hull under similar conditions. The insoluble nitrogen in neutral detergent, but soluble in acid detergent, is digestible, but it is slowly degraded in the rumen, whereas nitrogen in the form of insoluble nitrogen in acid detergent seems to be resistant and practically indigestible, and it is usually associated to lignin and other compounds of difficult degradation (Van Soest and Mason, 1991; Licitra et al., 1996), which may have contributed to the greater nitrogen excretion in the feces of the animals that received diets with coffee hull. The mean nitrogen value in the feces of 47.5 g/day observed for the diet without coffee hull was very close to the value of 48.6 g/day reported by Teixeira et al. (2007).

A quadratic effect was observed for the coffee hull levels (P<0.05) on the nitrogen balance, and a maximum value of 24.96 g was estimated for the level of 11.92% coffee hull in the concentrated feed. This performance was attributed to the recorded increases in the nitrogen excretion in the feces and decreases in the nitrogen excretion in the urine. In studies with Holstein heifers (pure and crossbred), receiving coffee hull in substitution for corn silage up to 21% of the total dry matter in the diet, Teixeira et al. (2007) did not report alterations in the nitrogen balance and recorded a mean value of 22.31 g/day.

The positive nitrogen balance for all the levels of coffee hull inclusion was an indication of an adequate balance between protein and energy in the diets. For the diet without coffee hull, it was observed that for the total of nitrogen ingested, approximately 29.14% was excreted in the feces, 60.74% in the urine and 10.12% was retained by the animal. In the diet with 26% coffee hull, however, 37.72% of the nitrogen was excreted in the feces, 55.83% in the urine and 6.45% was retained by the animal, that demonstrating a smaller use of the nitrogen contained in the coffee hull compared to the nitrogen present in corn.

Table 3 - Means for purine excretions and their derivatives in the urine and nitrogen microbial protein synthesis in function of coffee hull levels in the concentrated feed

Item	Diet				CV (%)	Regression equation
	0,0	8.75	17.5	26.25		
Allantoin, mmol/day	100.2	99.3	86.0	83.8	17.9	$\hat{Y} = 101.71 - 0.7153\,X$ ($r^2 = 0.88$)
Uric acid, mmol/day	14.2	14.7	11.4	10.7	37.3	$\hat{Y} = 12.76$
Total purine, mmol/day	114.4	114.0	97.4	94.5	18.7	$\hat{Y} = 116.54 - 0.8733\,X$ ($r^2 = 0.86$)
Allantoin/purine ration, %	87.6	87.1	88.3	88.7	---	Y = 87.9
Purine absorbed, mmol/day	103.8	104.3	84.6	82.5	23.5	$\hat{Y} = 106.33 - 0.9543\,X$ ($r^2 = 0.83$)
Microbial nitrogen, g/day	74.8	75.2	61.0	59.5	23.5	$\hat{Y} = 76.65 - 0.6879\,X$ ($r^2 = 0.83$)

CV = coefficient of variation.

A linear reduction (P<0.05) was observed for the urine allantoin excretions, total purine derivatives, absorbed purine and microbial production (Table 3) was estimated of 0.715, 0.873, 0.954 mmol/day and of 0.687 g/day, respectively, for each percentage unit of coffee hull added in the concentrated feed. However, the quantities of uric acid and the allantoin:purine derivative ratio were not changed by coffee hull addition. The lower nonfibrous carbohydrate intake and total digestible nutrient intake with the inclusion of coffee hull levels in the concentrated feed added to the smaller nutrient digestibility may have reduced the availability of protein and rumen energy of the diets which, according to Clark et al. (1992), are the nutritional factors that limit microbial growth at most. The study by Johnson et al. (1998) allowed this inference to be made, because these authors reported greater microbial nitrogen flow in diets with higher concentrations of degraded protein in the rumen and nonstructural carbohydrates. Therefore, it can be assumed that reduction in microbial protein synthesis in the rumen with the increase in coffee hull levels in the diet is a function of the smaller energy and protein availability in this ingredient compared to corn.

The mean values of allantoin and purine derivative observed in the animals submitted to the diet without coffee hull were close to the mean values of 94.96 × 105.77 mmol/day reported by Rennó et al. (2000) for heifers of different genetic groups receiving diets with 40% concentrate. The average allantoin excretion represented 87.9% of the purine derivatives, and it was higher than the mean value of 93.2% reported by Johnson et al. (1998). Nevertheless, values ranging from 83.7 to 86.8% were reported by Verbic et al. (1990) in experiments carried out with heifers.

Conclusions

The inclusion of coffee hull in the diet of dairy heifers in substitution for ground corn alters the nitrogen balance and increases the nitrogen excretion in the feces, which can be attributed to the high indigestible nitrogen contents present in this ingredient. Reduction in the microbial protein synthesis may explain, in part, the smaller weight gain of heifers fed diets with coffee hull.

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