

Feed restriction followed by realimentation in prepubescent Zebu females

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Abstract In the present study, the effect of restricting the feed intake for 77 days and subsequent compensatory growth for 50 days of Sindi females were evaluated. Eighteen animals with an initial age of 21 months and a mean weight of 211.7 kg were placed into three groups according to the following

alimentary regimens: feed ad libitum, feed restricted to 20 % dry matter, and feed restricted to 40 % dry matter. In the feed-restriction phase, the nutrient intake decreased ($P<0.001$) with an increase in the restriction level. As a consequence, the observed decrease in ingestion and serum concentrations of total protein, albumin and globulin, urea, glucose, calcium, and phosphorus were inversely proportional ($P<0.001$) to the restriction level. Significant differences in the nutrient intake and serum concentration were not observed in the realimentation phase ($P<0.05$). When animals in the control group reached the end of the feed-restriction phase, their weights ($P<0.05$) were similar to those in the 20 % restricted group, and both obtained a final weight that was greater than that of animals in the 40 % restricted group. In the feed-restriction phase, the control group had a similar mean daily weight gain ($P>0.05$) to animals in the 20 % restricted group and ($P<0.05$) 40 % restricted group. However, in the realimentation phase, the 40 % restricted group obtained greater weight gain rates ($P<0.05$), better food conversions, and partial compensatory gains. In particular, none of the restricted groups reached the final weight of the control group. In the feed-restriction phase, ingested nitrogen, nitrogen excreted in urine and feces, nitrogen balance and retained nitrogen decreased ($P<0.05$) with an increase in the restriction level. In the realimentation phase, none of the nitrogen balance variables were influenced by the restriction level ($P<0.05$). Females in the 40 % restricted group presented better food conversion rates and greater weight gains in the realimentation phase. Based on the animals' compensatory weight gain, a feed-restriction rate of 20 and 40 % can be adopted as a nutritional management practice for prepubescent Sindi females.

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Introduction

Dual-purpose Zebu breeds such as the Sindi are not often used in Brazil. The small producers' lack of technical knowledge (although they use empiricism in their rearing systems) and knowledge about scientific studies and the exploitation of unsuitable genotypes have resulted in high production costs and low sale prices for their corresponding products; thus, agricultural activity related to Sindi has not received significant attention in the livestock sector.

In northeastern Brazil, animals are typically subjected to alternating periods of food abundance and scarcity due to the quantity and quality of available food. The efficiency of food use is one of the main nutritional factors affecting the performance of cattle (Gonzaga Neto et al. 2011).

The biology of voluntary intake and animal performance response are complex, and their effects depend on the interaction of factors regarding the animal, diet, and environment; these factors are associated not only to the volume of roughage but also to the rate of roughage in the diet and/or level of concentrate (Neumann et al. 2007).

After a period of feed restriction and resumption of proper levels, a more intense growth rate is often observed. This phenomenon is often referred to as compensatory weight gain. Compensatory weight gain depends on a series of factors and their interactions, which leads to large variability in the magnitude of animal response to periods of feed restriction. Studies on metabolic and hormonal mechanisms may allow us to better understand the metabolic processes that occur when animals are submitted to periods of feed restriction and realimentation (Hoch et al. 2003). Thus, knowledge of the serum, urine, and ruminal concentrations of nutrients and the balance of their use can clarify the influence of these factors and their interactions on the manifestation of compensatory weight gain, especially when physiological factors are considered. In this context, the objective of the present study was to determine the effect of different feed-restriction levels and realimentation phases in prepubescent Sindi females.

Materials and methods

Experimental materials, procedures, and measurements

The experiment was carried out from June to October of 2008 at the Alagoinha-PB Experimental Station (Paraíba, Brazil). The research was carried out in two periods: 77 days of feed restriction and 50 days of realimentation. Eighteen Sindi females with an average age of 21 months and an average body weight of 211.7 kg at the start of feed restriction were used. The animals were placed into three groups of six animals such that two restriction levels were

generated in the feed-restriction phase and were compared to the group fed ad libitum. Namely, in group 1 (control), heifers were fed with a quantity of dry matter that met 100 % of the nutritional requirements for a gain of 700 g/day, according to the NRC (1996). In group 2, heifers were fed with enough dry matter to meet 80 % of the nutritional requirements of the control group. Lastly, in group 3, heifers were fed with a sufficient amount of dry matter to meet 60 % of the nutritional requirements of the control group. After restriction, the animals presented an average weight of 250.4 kg; then, the realimentation phase began and all of the animals received the same diet in the realimentation phase.

The ingredients used in the diets (elephant grass, cassava roots, corn meal, soybean meal, urea, and a mineral mixture) were processed and supplied in the form of complete feed. Samples of diet components were sampled every 2 weeks, and mixture composite samples, for each period. The orts of the feed were collected daily, weighed and sampled to form compost of animal residue/period, frozen, and analyzed afterwards.

The dry matter (DM), mineral matter (MM), total nitrogen, ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber contents were determined according to the method of Silva and Queiroz (2002). The crude protein (CP) content was obtained by multiplying the total nitrogen content by a factor of 6.25. The content of nonfibrous carbohydrates (NFC) was calculated according to the method of Weiss (1999) as $NFC (\%) = 100 - (\%NDFap + \%CP + \%EE + \%ash)$.

The chemical composition of the ingredients is shown in Table 1. Table 2 shows the proportion of ingredients and chemical composition of the experimental diets used in each period based on the dry matter content.

The quantity and orts of the feed were recorded daily to estimate the intake of the animals. In the restriction phase, the animals were weighed individually at 15-day intervals after a 14-h fast from solids. In the realimentation phase, the animals were weighed every 10 days, and the compensation index was assessed according to the methodology of Wilson and Osbourn (1960). This method quantifies the intensity of the weight compensation of the animals during realimentation according to the Equation $Index = (A - B) / A$, where A corresponds to the weight of animals that were not subjected to feed restriction during the first part of the experiment and B represents groups subjected to restriction (20 and 40 % in the present experiment) in one period and refed shortly afterwards.

In the restriction period and realimentation phase, blood samples were collected every 15 days, 3 h after feeding the heifers by puncturing the external jugular vein. A 10-mL test tube and a 10×25-mm sterilized needle were used without an anticoagulant to obtain the serum.

The samples were stored at room temperature and were centrifuged approximately 3 h later at 2,200×g for 20 min to

Table 1 Chemical composition of the ingredients in the experimental diets during the feed-restriction and realimentation periods

	Elephant grass	Cassava roots	Corn meal	Soybean meal
Feed restriction				
Dry matter ^a (DM)	26.00	38.30	88.90	85.55
Ash ^a	11.06	3.20	2.36	6.91
Crude protein ^a (CP) (N×6.25)	6.15	3.83	12.19	45.23
Ether extract ^a (EE)	2.17	1.27	5.47	1.19
Neutral detergent fiber ^a (NDF)	71.23	17.98	16.36	14.84
Neutral detergent fiber for ash and protein ^{a,b} (NDFap)	67.39	11.72	12.98	8.13
Acid detergent fiber ^a (ADF)	42.55	9.74	8.07	8.29
Nonfiber carbohydrates ^a (NFC)	13.23	79.98	67.00	38.54
Realimentation periods				
Dry matter ^a (DM)	26.89	24.49	86.26	86.45
Ash ^a	10.98	4.56	1.45	6.84
Crude protein ^a (CP) (N×6.25)	4.11	3.93	1013	44.41
Ether extract ^a (EE)	1.78	1.14	5.37	1.71
Neutral detergent fiber ^a (NDF)	78.65	58.06	13.55	14.82
Neutral detergent fiber for ash and protein ^{a,b} (NDFap)	71.74	49.20	9.87	10.73
Acid detergent fiber ^a (ADF)	49.26	37.33	4.54	9.99
Nonfiber carbohydrates ^a (NFC)	11.39	41.17	73.18	36.31

^a Dry matter percentage^b NDF was corrected for ash and protein (NDFap)**Table 2** Proportions and chemical compositions of the ingredients in the animals' experimental diet based on dry matter during the feed-restriction and realimentation periods

	Feed restriction (%)	Realimentation periods (%)
Ingredients		
Elephant grass	28.00	17.70
Cassava roots	35.31	–
Sugar cane	–	41.29
Corn meal	24.70	30.00
Soybean meal	9.10	7.52
Urea	1.28	1.49
Mineral mixture	1.61	2.00
Chemical composition		
Dry matter ^a (DM)	51.64	50.70
Ash ^a	5.43	4.77
Crude protein ^a (CP) (N×6.25)	13.82	12.93
Ether extract ^a (EE)	2.51	2.52
Neutral detergent fiber ^a (NDF)	31.69	43.07
Neutral detergent fiber for ash and protein ^{a,b} (NDFap)	26.95	36.78
Acid detergent fiber ^a (ADF)	18.10	26.24
Nonfiber carbohydrates ^a (CNF)	52.00	43.00

Percent composition: NaCl (64.57 %), KCl (23.07 %), NH₃SO₄ (7.69 %), ZnSO₄ (3.85 %), CuSO₄ (0.77 %), and CoSO₄ (0.015 %)^a Dry matter percentage^b NDF corrected for ash and protein (NDFap)

obtain the serum. The supernatant was aliquoted and stored at –20 °C in plastic microtubes until the biochemical analyses were performed.

The blood biochemistry was analyzed using LABTEST[®] biochemical kits, and biochemical determinations were made using an AMS[®] light spectrometer according to the following colorimetric methods: total protein (biuret), albumin (bromocresol green), urea (urease lab test), glucose (GOD-Trinder), cholesterol (Trinder enzymatic), calcium (methylthymol blue), and phosphorus (methylthymol blue).

Spot urine samples were taken by spontaneous miction on the 60th (feed restriction) and 115th (realimentation) day of the experiment approximately 4 h after the feed was supplied in the morning. In total, 10 L aliquots were diluted with 40 mL of 0.036 N H₂SO₄. The pH of the samples was adjusted to less than 3.0 to prevent bacterial destruction of urine purine bases and uric acid precipitation. The samples were stored at –20 °C until analysis for creatinine, urea, allantoin, and uric acid, according to methodology cited by Kozloski et al. (2005).

Urea and creatinine in the urine were analyzed by performing diacetyl methods, which were modified by the use of picrate and acidifier (Kit LABTEST[®]), respectively, using a light absorption spectrophotometer. Allantoin and uric acid were analyzed by performing the colorimetric method proposed by Fujihara et al. (1987).

The daily urine volume was estimated from the mean daily creatinine excretion, which was obtained in milligram

per kilogram body weight (BW) per day, and the creatinine concentration (in milligram per liter) of the urine spot sample. This volume was used to calculate the estimated daily urea, allantoin, and uric acid excretion of each animal.

The nitrogen compound balance was obtained by determining the difference between the total ingested nitrogen and the total excreted nitrogen in the feces and urine. The estimated daily excretion of N urea in urine was calculated by multiplying the urea urinary concentration by the estimated urinary volume and a factor of 0.466 (corresponding to the N content of urea). Total feces collection was carried out to determine the daily estimated N urea excretion. The total nitrogen content of the feces was determined according to the methodology described by Silva and Queiroz (2002).

The total amount of excreted purine derivatives was determined by calculating the sum of allantoin and uric acid urinary excretions. From these data, the content of absorbed microbial purines was calculated (X , in millimole per day) according to the following equation (Verbic et al. 1990): $Y = 0.85X + 0.385BW^{0.75}$, where 0.85 is the recovery of purines absorbed as purine derivatives in the urine and $0.385BW^{0.75}$ represents the endogenous contribution of purine excretion.

The intestinal flow of microbial nitrogen (N) compounds (Y , in gram N per day) was calculated as a function of absorbed purines (X , in millimole per day) according to the equation $Y = (70X) / (0.83 \times 0.134 \times 1,000)$, where 70 represents the N content of purines (in milligram N per millimole), 0.83 represents the digestibility of microbial purines, and 0.134 represents the N purine, the total N ratio in bacteria (Valadares et al. 1999).

Statistical analysis

A completely randomized design was used, and three treatments and six replicates per treatment were applied to obtain 18 plots. The metabolites were analyzed using the analysis plan of plots split in time, and the restriction level and collection time were used as the main and secondary plots, respectively. Thus, the performance and microbial protein production variables were submitted to an analysis of variance, and tests of means were conducted according to the GLM procedure in SAS (SAS 2005). The nutrient serum metabolic concentrations were analyzed using the MIXED procedure. The statistical models can be presented as follows: $Y_{ij} = \mu + r_i + e_{ij}$, where Y_{ij} is the observed value j of restriction level i , μ is the general mean, r_i is the effect of restriction level i , and e_{ij} is the random error associated with each observation. For the metabolites, time was considered in the subplot, and the model was described as follows: $Y_{ijk} = \mu + r_i + \alpha_{ij} + t_k + (rt)_{ik} + e_{ijk}$, where Y_{ijk} is the observed value j of restriction level i at time k , μ is the general mean, r_i is the effect of restriction level i , α_{ij} is the residual effect of the plots and can be characterized as error (a), t_k is the effect of the k th time level, $(rt)_{ik}$ is the effect of the

interaction of restriction level i and time level k , and e_{ijk} is the residual effect of the subplots, which can be characterized as error (b). In the presence of a treatment effect, the means of the treatments were compared using Tukey's test, and a significance level of 5 % was adopted for all of the tests.

Results

In the feed-restriction phase, the dry matter intake and its constituents decreased ($P < 0.001$) with an increase in restriction level, regardless of the unit (Table 3).

The DM intake as a function of the live weight of the animals in the 20 % feed-restriction group was in accordance with the NRC (1996) guidelines, which recommends an intake of 2.5 to 2.9 % LW for prepubescent females with a mean weight of 235 kg; however, the intake of the 40 % feed-restriction group was below the recommended values.

The intake of CP, neutral detergent fiber (NDF), NFC, and MM also decreased due to reduced dry matter ingestion. In the realimentation phase, significant differences ($P < 0.05$) in the intake of nutrients were not observed among groups. In the realignment phase, the food supply was similar among groups, and the intake of animals in different groups was similar.

Nitrogen intake, urine and fecal excretions, nitrogen balance, retained nitrogen, and microbial nitrogen before restriction were influenced ($P < 0.05$) by the restriction level (Table 4).

Nitrogen intake decreased ($P < 0.05$) due to the smaller crude protein intake of animals submitted to feed restriction. Nitrogen excretion in urine and feces was greater ($P < 0.05$) in animals fed ad libitum and decreased with an increase in the feed-restriction intensity, which was attributed to the magnitude of nitrogen intake.

The mean nitrogen urine excretion of the control group compared to the respective mean intake was equal to 11 % of ingested nitrogen. In the 20 and 40 % feed-restriction groups, urine excretion in relation to intake totaled 8.9 and 7.7 %, respectively. A decrease in the percent of excreted nitrogen in the urine was observed compared to the intake.

The nitrogen balance was also influenced ($P < 0.05$) by the restriction level, and the control, 20, and 40 % feed-restriction groups presented mean values of 302, 262, and 236 g/day, respectively. These results were related to an increase in nitrogen intake and nitrogen excretion in the feces and urine. The percentage of retained nitrogen in relation to ingested nitrogen was also influenced ($P < 0.05$) by the diet, and the most restricted group presented greater nitrogen retention rates than the control and 20 % feed-restriction groups, which did not differ.

In the restriction phase, a decrease in microbial nitrogen production was observed due to feed restriction ($P < 0.05$),

Table 3 Mean, coefficient of variation (CV), and probability (P) of the daily intake of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), nonfibrous carbohydrates (NFC), and ash in Sindi females as functions of the feed-restriction level and realimentation growth period

Intake of nutrients	Levels of growth restriction			%CV	P value
	0 %	20 %	40 %		
Feed restriction					
Dry matter (kg/day)	8.18 a	6.13 b	4.60 c	12.40	<0.0001
Dry matter (%BW)	3.40 a	2.65 b	2.09 c	5.75	<0.0001
Dry matter (g/BW ^{0.75})	133.85 a	103.55 b	80.69 c	7.22	<0.0001
Crude protein (kg/day)	1.17 a	0.96 b	0.79 c	12.22	<0.0001
Crude protein (%BW)	0.49 a	0.41 b	0.33 c	5.04	<0.0001
Crude protein (g/BW ^{0.75})	26.07 a	25.27 b	24.55 c	6.66	<0.0001
Neutral detergent fiber (kg/day)	3.12 a	2.52a	1.89 b	12.87	<0.0001
Neutral detergent fiber (%BW)	1.29 a	1.09 b	0.86 c	6.74	<0.0001
Acid detergent fiber (kg/day)	1.23 a	1.11 a	0.83 b	12.25	<0.0001
Ether extract (kg/day)	0.26 a	0.19 b	0.14 b	13.67	<0.0001
Nonfiber carbohydrates (kg/day)	3.28 a	2.32 b	1.73 c	12.56	<0.0001
Ash (kg/day)	0.46 a	0.37 b	0.28 c	12.11	<0.0001
Realimentation periods					
Dry matter (kg/day)	7.82	7.93	7.32	8.75	0.4762
Dry matter (%BW)	3.43	3.52	3.42	15.01	0.8234
Dry matter (g/BW ^{0.75})	133.14	136.56	130.97	12.33	0.7739
Crude protein (kg/day)	1.01	1.03	0.99	6.57	0.4596
Crude protein (%BW)	0.49	0.51	0.54	13.13	0.6117
Crude protein (g/BW ^{0.75})	19.02	20.03	20.70	10.44	0.5362
Neutral detergent fiber (kg/day)	3.23	3.36	3.08	11.58	0.5769
Neutral detergent fiber (%BW)	1.42	1.49	1.44	16.71	0.8155
Acid detergent fiber (kg/day)	2.06	2.25	2.39	13.37	0.4526
Ether extract (kg/day)	0.20	0.21	0.21	8.04	0.3437
Nonfiber carbohydrates (kg/day)	3.79	3.74	3.52	5.51	0.2195
Ash (kg/day)	0.48	0.49	0.45	8.96	0.5411

Means followed by the same letter were not significantly different in the Tukey's test at a probability level of 5 % ($P < 0.05$)

and the highest restriction level (40 %) presented the smallest values ($P < 0.05$). In the realimentation phase, the feed-restriction level ($P < 0.05$) did not have an effect on the nitrogen balance variables. Compared to the respective mean intake, the mean nitrogen urine excretion of the control, 20 and 40 % feed-restriction groups totaled 18.6, 19, and 18.3 % of the intake, respectively. These results were expected because the animals ingested the same diet in this phase.

The severity of restriction influenced ($P < 0.05$) the final weight and daily mean weight gain of the animals (Table 5). The control group and group submitted to 20 % feed restriction had a higher final weight than the 40 % feed-restriction group. Compared to animals fed ad libitum, a 20 % restriction did not influence ($P < 0.05$) the final weight and mean daily gain of the animals, and similar results were observed with dry matter intake. The control group reached the end of the feed-restriction phase with a weight that was 12 % greater than the final weight of animals receiving feed restricted by 40 %. The control group and 20 % restriction group presented significantly higher mean weight gains ($P < 0.001$) than the group receiving 40 % less feed

(%DM). The mean weight gain of animals in the control group was 35 % greater than that of the 40 % feed-restriction group.

In the realimentation phase, the daily mean weight gain of animals submitted to 40 % feed restriction was greater ($P < 0.05$) than that of the control group, and neither group differed from the 20 % feed-restriction group. The final weight of animals in the control group did not differ ($P < 0.05$) from that of the animals submitted to 20 % feed restriction, and both groups had a higher final weight ($P < 0.05$) than that of the 40 % feed-restriction group. In spite of the greater weight gain, animals subjected to 40 % feed restriction had a lower live weight at the end of the experiment. Analysis of the compensation index showed that all of the experimental groups presented compensation rates between 93 and 99 %, and significant differences ($P < 0.05$) among restriction levels were not observed. Both restricted groups (20 and 40 %) presented high compensation rates.

Although significant differences ($P < 0.05$) among groups were not observed, animals in the group submitted to 20 % feed restriction presented values that were closer to total

Table 4 Mean, coefficient of variation (CV), and probability (P) of ingested nitrogen, fecal and urinary nitrogen excretion, nitrogen balance, and microbial nitrogen compounds (N_{mic}) of Sindi females as functions of the feed-restriction level and realimentation growth period

Parameters	Level of growth restriction			%CV	P value
	0 %	20 %	40 %		
Feed restriction					
Ingested nitrogen, g/day	731.25 a	600.00 b	493.75 c	8.34	0.0123
Urinary nitrogen, g/day	80.43 a	53.40 b	38.01 c	22.67	0.0446
Fecal nitrogen, g/day	348.07 a	283.80 b	219.22 c	17.25	0.0234
Nitrogen balance, g/day	302.75 a	262.80 b	236.52 c	20.45	0.0269
Retained nitrogen, % ^a	41.40 b	43.80 b	47.90 a	10.21	0.0340
Microbial nitrogen, g/day	269.44 a	199.37 b	172.56 c	6.58	0.0001
Realimentation period					
Ingested nitrogen, g/day	631.25	643.50	618.75	6.70	0.5709
Urinary nitrogen, g/day	117.41	122.26	113.23	24.17	0.2956
Fecal nitrogen, g/day	328.45	310.81	306.90	17.22	0.8131
Nitrogen balance, g/day	185.39	210.43	198.62	18.66	0.9888
Retained nitrogen, % ^a	29.36	32.70	32.10	11.46	0.7394
Microbial nitrogen, g/day	288.42	297.20	289.94	3.14	0.0544

Means followed by the same letter were not significantly different in the Tukey's test at a probability level of 5 % ($P < 0.05$)

^aNitrogen balance/ingested nitrogen

compensation. In particular, although a 20 % feed restriction was applied, the weights and weight gains ($P < 0.001$) of restricted animals at the end of the experiment were similar to those of the control group, who were supplied with sufficient feed to obtain a gain of 700 g/day.

Food conversion in the restriction phase was greater ($P < 0.05$) in the control group and was not significantly different ($P < 0.05$) among groups submitted to feed restriction. In the realimentation phase, the 40 % feed-restriction

group presented better ($P < 0.05$) food conversion rates, which was a reflection of compensatory gain.

In the realimentation phase, the serum concentrations of total protein, albumin, globulin, urea, glucose, calcium, and phosphorus were inversely proportional ($P < 0.001$) to the restriction level (Table 6). An increase in the urea concentration ($P < 0.05$) from the feed-restriction period to the compensatory gain period and an increase in the glucose concentration after realimentation were observed. The

Table 5 Mean, coefficient of variation (CV), and probability (P) of the initial weight (in kilogram per day), final weight (in kilogram per day), mean daily weight gain (in kilogram per day), and food conversion and

compensation index (in percent) as functions of the live weight and metabolic weight of Sindi females submitted to different periods of feed restriction and realimentation

Variable	Levels of growth restriction			%CV	P value
	0 %	20 %	40 %		
Feed restriction					
Weight initial live (kg)	211.33	214.67	207.50	8.46	0.3552
Weight final live (kg)	264.90 a	253.13 a	233.27 b	11.58	0.0319
Average daily gain (kg/day)	0.699 a	0.616 a	0.460 b	16.59	0.0463
Feed conversion ^a	11.70 a	9.95 b	10.00 b	14.95	0.0119
Realimentation period					
Weight final live (kg)	289.60 a	283.53 a	267.60 b	10.83	0.0416
Average daily gain (kg/day)	0.598 b	0.608 a, b	0.686 a	14.64	0.0219
Feed conversion ^a	15.95 b	13.04 b	10.06 a	26.17	0.0251
Compensation in body weight (%)	–	98	93	8.37	0.5667
Compensation in weight ^{0.75} (%)	–	99	94	7.54	0.6754

Means followed by the same letter were not statistically different in the Tukey's test at a probability level of 5 % ($P < 0.05$)

^a FC in kilogram DM per kilogram ADG

Table 6 Mean, coefficient of variation (CV), and probability (P) of the serum concentration of total protein (in gram per deciliter), albumin (in gram per deciliter), globulin (in gram per deciliter), urea (in milligram per deciliter), glucose (in milligram per deciliter), calcium (in milligram per deciliter), and phosphorus (in milligram per deciliter) in Sindi females submitted to different feed-restriction and realimentation periods

Metabolites	Level of growth restriction			%CV	P value
	0 %	20 %	40 %		
Feed restriction					
Serum total protein (g/dL)	7.00 a	5.76 b	4.64 c	16.86	<0.0001
Serum albumin (g/dL)	3.35 a	2.89 b	2.09 c	11.31	<0.0001
Serum globulin (g/dL)	3.65 a	2.87 b	2.55 c	27.17	<0.0001
Serum urea (mg/dL)	54.48 a	49.56 b	43.38 c	12.68	<0.0001
Serum glucose (mg/dL)	60.00 a	57.07 b	54.01 c	16.16	0.0002
Serum calcium (mg/dL)	10.74 a	9.51 b	8.56 c	10.53	<0.0001
Serum phosphorus (mg/dL)	5.50 a	4.94 b	4.37 c	10.55	<0.0001
Realimentation period					
Serum total protein (g/dL)	7.08	6.90	6.76	3.71	0.1307
Serum albumin (g/dL)	3.47	3.56	3.36	8.82	0.5356
Serum globulin (g/dL)	3.61	3.33	3.39	13.70	0.5813
Serum urea (mg/dL)	52.45	53.31	55.11	7.69	0.1481
Serum glucose (mg/dL)	61.65	60.51	60.31	12.20	0.3779
Serum calcium (mg/dL)	10.86	10.61	10.25	6.66	0.2141
Serum phosphorus (mg/dL)	5.40	5.56	5.34	6.94	0.2416

Means followed by the same letter were not significantly different in the Tukey's test at a probability level of 5 % ($P < 0.05$)

serum concentration of calcium and phosphorus decreased ($P < 0.001$) with an increase in the feed-restriction level.

Discussion

The intake of the animals was regulated with respect to the amount of DM ingested by the control group (intake ad libitum). The feed intake of the animals was inversely proportional to the severity of restriction. Tolla et al. (2003) and Gonzaga Neto et al. (2011) also observed reduced feed intake according to the severity of the restriction level. Restriction may or may not result in weight gain compensation and increased food intake; however, in the realimentation period, the animals resumed normal nutrient intake. In the present experiment, restriction was quantitative.

Due to the reduction in nutrient ingestion, a decrease in the serum concentration of most of the studied nutrients was observed. Possible alterations in metabolism due to reduced food intake include a decrease in the content of propionic acid in hepatic tissue, which causes changes in neoglucogenesis due to the use of glycerol, and a decrease in the amount of lactate and some amino acids, which reduces glycemia. Similar to the results of the present experiment, Hoch et al. (2003) detected decreased glucose levels when restriction was prolonged. As the serum glucose level decreased, the animal's fatty acids mobilized from fat tissue during feed restriction to avoid using protein and energy. However, if the animal is subjected to severe and gradual energy deficiency, a large mobilization of muscular protein occurs in an attempt to maintain energetic homeostasis, especially the blood glucose

content. In this case, the animal stops depositing muscle tissue, and muscle development is inhibited.

The increase in the urea concentration ($P < 0.05$) observed from period to the compensatory gain period reflected an increase in hepatic synthesis, which resulted from an increase in microbial nitrogen due to enhanced ruminal production because of the greater amino acid input in the liver and the feed (Huntington et al. 2004). The increase in the glucose concentration observed after realimentation occurred as a result of greater ruminal propionic acid production, which was associated with a greater concentration of nutrient energy. Because the diet was formulated with correctly correlated levels to prevent antagonism, the observed reduction in serum levels was attributed to a decrease in the ingestion of minerals due to feed restriction. Specifically, a significant reduction ($P < 0.05$) in the mineral matter intake was observed as a function of the restriction level. Prolonged deficiency of these two minerals can decrease the body size of the animal and alter the beginning of reproductive life (Taylor et al. 2008). In a study conducted by Hoch et al. (2003), the adaptation of energetic and protein metabolism allowed the animal to make the best use of foods and conserve food in the first phase of realimentation, enabling the organism to become more efficacious for nutrient use.

When imbalances caused by the absence of a nutrient are severe or even moderate and persistent, the animal exhausts its body reserves and disease occurs. In the present experiment, the animals withstood the restriction period, and metabolic disturbances in the animals or animals in pathological states were not observed.

According to Wilson and Osbourn (1960), an animal's growth can be delayed if an element is missing from its diet and energy and protein limit weight gain. The smaller nutrient intake observed in the present experiment was reflected in reduced serum contents of protein and glucose, which resulted in smaller weight gains. In addition, the lowest final weights corresponded to animals subjected to severe feed restriction. Specifically, more nutrients are available for tissue deposition when feed intake is greater than that required for maintenance. Thus, the proportion of nutrients used for maintenance is smaller when feed is restricted.

Hoch et al. (2003) reported that needy animals gain less weight because they reduce their basic nutritional requirements to decrease their metabolic rate and attempt to save and use nitrogen for energy production, which could explain the observed decrease in the serum volume of protein in cattle submitted to feed restriction. According to Ryan (1990), compensation is complete when higher gain rates of compensatory growth can fully compensate for poorer performance in the feed-restriction period. In contrast, partial compensation is observed when higher gain rates of compensation are not sufficient to recover what was not gained in the feed-restriction period. In the present experiment, none of the animals presented a value of 100 %, which would correspond to total compensation.

According to Yan et al. (2007), a decrease in dry matter and nitrogen intake can negatively influence dietetic and microbial nitrogen flows, and nitrogen excretion in the urine and feces is directly related to the concentration of crude protein in the diet and nitrogen ingestion by the animal. One of the most effective strategies for reducing nitrogen excretion is to manipulate its concentration in the diet. Thus, feed restriction can be an efficient tool for reducing nitrogen elimination in feces and urine, as long as it does not damage animal performance.

The positive nitrogen balance indicated that protein was retained in the animal, which provided conditions such that weight loss did not occur in the experimental animals (Zanton and Heinrichs 2008). When energy requirements are met, protein retention in the animal provides conditions for weight gain. Taylor-Edwards et al. (2009), Pereira et al. (2007), and Valkeners et al. (2004) observed that greater ingestion of nitrogen compounds resulted in greater nitrogen retention in the animal.

Reduced microbial protein production was related to the fact that the animals presented similar DM intakes (in kilogram per day), as reported by Yan et al. (2007). Thus, animals that ingested more nutrients presented greater ruminal microorganism synthesis. According to Valkeners et al. (2004), nitrogen retained in the rumen also plays an important role because it becomes available for microorganisms and allows the continuous synchronization of nitrogen and energy as substrates.

The balance between the nitrogen and energy supply for ruminal microorganisms may be a mechanism for increasing the capture of ruminal degradable nitrogen and improving microbial growth (Pereira et al. 2007). Therefore, an increase in available nutrients due to greater ingestion also promotes greater efficiency in microbial protein synthesis, which improves performance. Thus, animals that ingested more nutrients (control group) presented greater ruminal microorganism synthesis.

Feed restriction decreased food ingestion, nutrient serum levels, and the availability of ammoniacal nitrogen but did not damage microbial microflora, although animal performance was reduced.

Conclusions

In spite of the 20 % feed restriction, prepubescent Sindi females maintained dry matter intakes that were in accordance with the recommendations of the NRC (1996), and the daily weight gain and final weights of the animals in the 20 % feed-restriction group were not less than that of animals fed ad libitum. Females submitted to 40 % feed restriction obtained better food conversion rates and higher weight gains in the realimentation phase. Therefore, feed-restriction levels (20 %) can be adopted as nutritional management practices for prepubescent Sindi females, and high partial compensation rates can be obtained.

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