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Plasma urea levels on reproductive parameters of wool-less rams (*Ovis aries*, Linnaeus, 1758) *

Nível plasmático de uréia sobre parâmetros reprodutivos de machos ovinos deslanados (*Ovis aries*, Linnaeus, 1758)

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SUMMARY

The effect of increased plasma urea levels on reproductive parameters of Santa Ines Brazilian breed rams (*Ovis aries*) was determined during two phases, 60 days in length each. During the first phase, 18 animals divided into three blocks according to scrotal circumference were submitted to the following treatments: diet A_1 - with nitrogen levels according to ARC (1980); diet B_1 - nitrogen levels 133% higher than A_1; and diet C_1 - nitrogen levels 166% higher than A_1. During the second phase, 12 animals divided into two groups received diet A_2 - with nitrogen levels according to ARC (1980), and diet B_2 - nitrogen levels 200% higher than diet A_2, respectively. The increase in protein equivalents was obtained by adding 20, 40 and 60 g of feed grade urea to treatments B_1, C_1 and B_2, respectively. Semen was collected daily using an artificial vagina and analyzed twice a week for progressive motility; vigor, whirling, density, pH, concentration, sperm morphology, ejaculated volume and total sperm per ejaculate. Urea concentration was determined weekly in semen and plasma. Urea level increased in plasma and semen ($p < 0.0001$) during both phases but did not affect the parameters studied.

UNITERMS: Urea; Semen; Reproduction; Rams; Feed Ration.

INTRODUCTION

The achievement of high levels of fertility and prolificacy in sheep flocks relies not only upon the female members but also upon their male consorts[1].

Diets with high levels of crude protein, nitrogen compounds or lack of a ruminal substrate for complete transformation of ammonia into bacterial protein may increase rumen and consequently plasma urea concentration. Plasmatic urea can reach the seminiferous tubules[26,34] and the presence of testicular transporters[39] for urea suggests that it may play a role in spermatogenesis[39], despite increased plasma urea concentration in humans due to renal insufficiency[17,18,19,20,24,25,28,31], and in dairy cows[7,8,9,12,13,14,15,21,22] due to high levels of nitrogen compounds in the diet, are considered to predispose to reduced fertility. Impaired reproduction in bulls[6,10] reported with increased dietary nitrogen compounds was not always confirmed neither in bulls[4,32,33,38] nor in rams[29,30,38].

The present study investigated the effect of increased plasma urea levels on the reproductive parameters of rams.

MATERIAL AND METHOD

Hybrid wool-less Santa Ines rams aged 11 to 18 months and weighing 33.1 to 41.4 kg (live weight) individually pen housed indoors were submitted to daily sperm collection with an artificial vagina kept at 46°C, using females as manikins, from May to October 1996 at the Center of Biotechnology in Animal Reproduction, Pirassununga (BR), located at 21°59' South latitude and 47°33' West longitude of Greenwich. Ambient temperature ranged from 12.4°C to 25.0°C and daytime duration from 10 h and 56 min to 12 h and 44 min, during the experimental period.

The treatments consisted of isoenergetic diets containing different nitrogen levels, in two different phases, as follows.

During phase 1 (May to July), 18 animals were used

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in a generalized randomized block design[16] with three treatments and three blocks and treatment replicates within blocks. The animals were assigned to the different blocks according to scrotal circumference. Treatments were structured with three increasing levels of dietary nitrogen: diet A_1 - ration containing nitrogen levels according to[2], or 0 g of urea; diet B_1 - a 133% increase in nitrogen compared to diet A_1 or 20 g urea, gradually increased and diet C_1 - a 166% increase in nitrogen compared to diet A_1, or 40 g of urea, gradually increased. Cornmeal was used on all diets, serving as a vehicle for urea in diets B_1 and C_1.

During phase 2 (August to October), 12 animals previously assigned to treatments A_1 and C_1 (phase 1) were kept in phase 2 and renamed as treatments A_2 and B_2. The animals previously assigned to treatment B_1 were discarded. A fully randomized design was used[16], with two treatments and six replicates per treatment. The treatments consisted of diet A_2 - ration with a nitrogen level according to[2], or 0 g of urea and diet B_2, a 200% increase in protein equivalents compared to diet A_2, or 60 g urea, gradually increased from diet C_1. Cornmeal was added to all diets, serving as vehicle for urea in diet B_2.

The composition of all the rations is shown in Tab. 1. The parameters started to be analyzed only after the desired amount was reached in each ration.

The following seminal parameters were analyzed twice a week: progressive straight motility[27], vigor[27], whirling[27], density[11], volume[11], sperm concentration[27], total number of ejaculated spermatozoa[11], pH, measured with a portable digital potentiometer (CORNING® - EUA) soon after collection, and sperm morphology[3,5], using an interference contrast microscope (JENAVAL® Germany). Semen and blood plasma aliquots were collected weekly and stored at –20°C for later urea determination by the colorimetric enzymatic method using a commercial kit (CELM®- BR). For semen urea determination, the aliquot was first submitted to deproteination[11].

Scrotal circumference was measured (cm) at 15 days intervals using a specific tape (Lane Manufacturing Inc.® EUA) and testis and epididymis weight were determined after surgical removal of the gonads using an analytical scale, at the end of phase two.

Data was analyzed statistically using the Statistical Analysis System software[36] after residue normality tested by the Shapiro-Wilk test (Proc UNIVARIATE) and variances compared by the F test. Phase 1 data for plasma and seminal urea, seminal parameters and scrotal circumference were submitted to analysis of variance by the GLM procedure (PROC GLM) and means separation was achieved by orthogonal contrasts. The effects of treatment for morphological sperm alterations were determined by non-parametric rank analysis using the Kruskal-Wallis test. Phase 2 data for plasma and seminal urea, seminal parameters, scrotal circumference, testis and epididymis weight were submitted to analysis by the *t*-test (PROC TTEST). For data concerning morphological sperm alterations, the effect of treatment was determined by non-parametric rank analysis using the Wilcoxon 2-sample test. The level of significance was set at 5% in all analyses.

RESULTS, DISCUSSION AND CONCLUSION

There was an increase ($p < 0.0001$) in plasma and semen urea concentration (Tab. 2) that had no effect on the semen parameters analyzed (Tabs. 3 and 4), as also described by Thompson *et. al.*[38], who detected no difference in semen quality or fertility of bulls and rams. The urea increase also had no effect on testis and epididymis weight and on scrotal circumference (Tab. 5) according to Rocha *et. al.*[32]. However, these results were in contrast to those reported by Branton *et. al.*[6] and Castillo *et. al.*[10], who observed a reduction of these parameters in bulls with increased amount of dietary protein.

Table 1
Composition of daily amount ingredients and nutrients during phases 1 and 2, Pirassununga, 1996.

	Phase 1			Phase 2	
	A_1	B_1	C_1	A_2	B_2
Ingredient consumption (g)					
Pellet mixture[a]	650.4	650.4	650.4	650.4	650.4
Coast cross hay (IFN 1-00-716)	534.6	534.6	534.6	534.6	534.6
Cornmeal (IFN 4-21-018)	172.4	172.4	172.4	172.4	172.4
Urea	-	20	40	-	60
Dry matter nutrients					
Metabolizable energy (Mcal/kg)	2.10	2.08	2.07	2.10	2.00
Crude protein (%)	11.9	15.9	19.8	11.9	23.7
Nitrogen (%)	1.9	2.5	3.2	1.9	3.8
Increase in nitrogen (%)	-	133	166	-	200

[a] Composition (% of dry matter) of the pellet mixture: Coast cross hay (IFN 1-00-716), 30.0%; Soybean meal (IFN 5-04-604), 7.0%; Cornmeal (IFN 4-21-018), 61.5%; Mineral supplement, 1.5%.

Table 2

Urea concentration (mg/dl) in plasma and semen (mean ± sem), coefficient of variation (C.V.), statistical probability, regression equation and coefficient of determination (R^2), Pirassununga, 1996.

	Treatment			C.V.(%)	Probability[b]		Equation	R^2
Phase 1	A_1	B_1	C_1		Linear	Deviation		
Plasma	18.3 ± 0.9	34.4 ± 2.2	49.8 ± 1.7	43.56	0.0001	0.1232	Y= 18.39 + 0.31X	0.75
Semen	32.5 ± 3.7	51.4 ± 4.2	60.5 ± 3.0	30.31	0.0001	0.0048	Y= 32.09 + 0.38X-0.001X^2	0.52
	Treatment			C.V.(%)	Probability[c]			
Phase 2	A_2		B_2					
Plasma	26.6 ± 0.8		77.2 ± 1.7	48.58	0.0001			
Semen	58.3 ± 3.1		106.1 ± 2.1	33.84	0.0001			

[a] SEM, Standard error of the mean; [b] P values for linear regression and deviations; [c] P values for the *t*-test.

Table 3

Semen parameters (mean ± sem[a]), coefficient of variation (C.V.), statistical probability, of rams fed with increasing nitrogen levels, Pirassununga, 1996.

	Treatment			C.V.(%)	Probability[b]	
Phase 1	A_1	B_1	C_1		Linear	Deviation
Progressive straight motility (%)	67.5 ± 0.8	69.8 ± 0.8	69.8 ± 1.0	9.64	0.08	0.29
Vigor	3.2 ± 0.05	3.4 ± 0.04	3.3 ± 0.04	11.38	0.19	0.10
Whirling	3.4 ± 0.05	3.7 ± 0.03	3.4 ± 0.12	16.36	0.84	0.04
pH	7.4 ± 0.1	7.2 ± 0.1	7.4 ± 0.2	4.39	0.81	0.22
Density (g/ml)	1.06 ± 0.3	1.06 ± 0.3	1.00 ± 0.3	18.48	0.06	0.26
Volume (ml)	1.0 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	27.78	0.37	0.94
Concentration (x10^9 sperm/ml)	2.40 ± 0.15	2.62 ± 0.16	2.09 ± 0.15	38.55	0.34	0.19
Sperms/ejaculation (x10^9)	2.34 ± 0.51	2.46 ± 0.52	1.83 ± 0.45	45.96	0.20	0.27
Phase 2	Treatment			C.V.(%)	Probability[c]	
Progressive straight motility (%)	66.3 ± 1.1		68.2 ± 0.7	9.21	0.29	
Vigor	3.4 ± 0.1		3.3 ± 0.1	12.06	0.77	
Whirling	3.3 ± 0.1		3.5 ± 0.1	18.37	0.73	
pH	6.4 ± 0.1		6.4 ± 0.1	4.37	0.82	
Density (g/ml)	0.94 ± 0.04		1.06 ± 0.01	5.48	0.60	
Volume (ml)	1.1 ± 0.04		1.2 ± 0.04	23.11	0.28	
Concentration (x10^9 sperm/ml)	4.07 ± 0.16		4.26 ± 0.13	23.82	0.46	
Sperm./ejaculation (x10^9)	4.28 ± 0.28		4.78 ± 0.23	38.22	0.38	

[a] SEM, Standard error of the mean; [b] P values for linear regression and deviations; [c] P values for the *t*-test.

Table 4

Sperm morphology alterations (mean ± sem[a]), coefficient of variation (C.V.) and statistical probability of rams fed with increasing nitrogen levels, Pirassununga, 1996.

	Treatment			C.V.(%)	Probability[b]
Phase 1	A_1	B_1	C_1		
Morphology alteration (%)	12.1	12.7	12.0	13.00	0.4990
	Treatment			C.V.(%)	Probability[c]
Phase 2	A_2		B_2		
Morphology alteration (%)	10.1		7.1	67.00	0.122

[a] SEM, Standard error of the mean; [b] P values for Kruskall Wallis rank test; [c] P values for Wilcoxon 2 - sample rank test.

Table 5

Testis plus epidydimis weight (mean ± sem[a]) coefficient of variation (C.V.) and statistical probability, Pirassununga , 1996.

	Treatments[a]		C.V. (%)	Probability[b]
	A_2	B_2		
Testis + right epidydimis ± SEM	159.4 ± 9.9	168.9 ± 9.8	14.5	0.5892
Testis + left epidydimis ± SEM	155.3 ± 15.5	170.6 ± 9.4	15.5	0.4040

[a] SEM, Standard error of the mean; [b] P values for the *t* - test.

The results were also in contrast to remarks in which were observed gonadal degeneration and infertility, with reduced sperm production and loss of libido, attributed to the increase in plasma urea levels[17,18,19,20,24,28,31] in human patients with renal insufficiency. The lack of effects in these rams' semen parameters, despite the urea increase in plasma and semen urea levels may be associated to levels not high enough to become harmful, since plasma urea levels remained below the broad range considered to be normal in rams by different authors, e.g., from 17 to 42[23] mg/dl or 43 to 75 mg/dl[35,37].

RESUMO

Estudou-se o efeito de níveis aumentados de uréia plasmática sobre parâmetros reprodutivos de machos ovinos (*Ovis aries*) deslanados da raça Santa Inês, em duas fases de 60 dias cada. Durante a primeira fase, 18 animais divididos em três blocos de acordo com perímetro escrotal foram submetidos aos seguintes tratamentos: dieta A_1 – com níveis de nitrogênio de acordo com ARC (1980); dieta B_1 – com níveis de nitrogênio 133% maiores que dieta A_1; e dieta C_1 – níveis de nitrogênio 166% maiores que dieta A_1. Durante a segunda fase, 12 animais divididos em dois grupos receberam as dietas: A_2 – com níveis de nitrogênio de acordo com ARC (1980) e dieta B_2 – níveis de nitrogênio 200% maiores que dieta A_2. O aumento no equivalente protéico foi obtido com adição de 20, 40 e 60 g de uréia aos tratamentos B_1, C_1 e B_2, respectivamente. Foi coletado sêmen diariamente com vagina artificial e duas vezes por semana foram analisados: motilidade retilínea e progressiva, vigor, turbilhonamento, densidade, pH, concentração, morfologia espermática, volume ejaculado e total de espermatozóides por ejaculado. A concentração de uréia no plasma e no sêmen foi analisada semanalmente. O aumento ($p < 0,0001$) de uréia no sêmen e no plasma durante ambas as fases não afetou os parâmetros estudados.

UNITERMOS: Uréia; Sêmen; Reprodução; Carneiros; Rações.

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