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Behaviour of confined sheep fed with different concentrate sources

[*Comportamento ingestivo de ovinos confinados alimentados com diferentes fontes de concentrado*]

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ABSTRACT

In this study we examined the effects of different feed concentrates on sheep behaviour. Our hypothesis was that citric pulp would stimulate rumination and be capable of replacing other concentrates traditionally used for feeding in confinement, to reduce the risk of urolithiasis. Ten adult Santa Inês sheep were distributed in a Latin square with five different diets, one control diet with 80% hay and 20% commercial feed and four diets containing 30% coast-cross hay and 70% of the following concentrates: pelleted citrus pulp, citrus pulp meal, cornmeal or wheat bran. After 21d of adaptation to each one of the five diets, the sheep were visually monitored for 24 h at 3 min intervals to record the time spent ruminating, time spent eating and time spent resting; the animals' positions (standing or lying down) were also noted. Daytime was considered to be from 06:00h to 18:00h. The data were evaluated using ANOVA, with Tukey post-hoc test or throughout Two-sample T test for circadian and position assessment. Citrus pulp diets resulted in time spent ruminating similar to the control diet (601, 590 and 669 min, respectively), but greater ($P<0.05$) than the cornmeal group (421min), which showed that citrus pulp generated effective rumination. The estimated saliva production in the control diet (26L) was greater than in the other groups, and was greater in the citrus pulp groups (24L/d) than cornmeal (21L/d). Feeding with cornmeal led to shorter time spent eating and time spent ruminating than all other diets. The sheep had higher time spent resting at night when fed concentrates ($P<0.05$). For all diets, about 90% of the time spent ruminating occurred with the animals lying down. Pelleted citrus pulp, citrus pulp meal and to a lesser degree wheat bran, led to adequate time spent ruminating. The use of citrus pulp can act as a preventive management measure to reduce the incidence of urolithiasis in sheep flocks.

Keywords: feed behaviour, citric pulp, rumination, urolithiasis, cornmeal

RESUMO

No presente estudo, avaliaram-se os efeitos da alimentação de diferentes concentrados sobre o comportamento de ovinos. A hipótese é a de que a polpa cítrica estimularia a ruminação e reduziria o risco de ocorrência de urolitíase, podendo substituir outros concentrados. Dez ovinos adultos, mestiços da raça Santa Inês, foram distribuídos em um quadrado latino com cinco tratamentos, sendo quatro destes contendo dietas com 30% de feno de capim coast-cross *e 70% dos seguintes concentrados: polpa cítrica peletizada, polpa cítrica farelada, fubá de milho e farelo de trigo, e uma dieta controle com 80% de feno e 20% de ração comercial peletizada. Após 21 dias de adaptação, a cada dieta os animais foram colocados em monitoramento visual, com registro de atividade a cada três minutos, durante 24 horas consecutivas, por meio do qual foi identificado o tempo gasto na ruminação, na ingestão de alimentos e em repouso. A posição dos animais (em pé ou deitados) também foi observada. O período diurno foi considerado entre seis e 18 horas. Para comparação entre os tratamentos, os dados foram avaliados por meio de ANOVA e do teste de Tukey. Para a avaliação circadiana e entre as posições, foi utilizado o teste t de Student. Dietas com polpa cítrica promoveram tempo de ruminação semelhante aos do grupo de controle (601, 590 e 669 min, respectivamente), mas superior ao grupo alimentado com fubá de milho (421min). A produção de saliva estimada no grupo controle (26L/d) foi maior*

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do que nos demais grupos, e os grupos com polpa cítrica tiveram maior produção de saliva do que o grupo com fubá de milho (21L/d). Ovinos em dietas ricas em concentrados descansam mais durante a noite. Em todas as dietas, cerca de 90% da ruminação ocorreu com os animais deitados. A polpa cítrica peletizada e a farelada, e em menor grau o farelo de trigo, promoveram adequadamente a ruminação. Este concentrado pode ser utilizado como medida preventiva visando diminuir a incidência de urolitíase em rebanhos ovinos.

Palavras-chave: comportamento alimentar, polpa cítrica, ruminação, urolitíase, fubá de milho

INTRODUCTION

Urolithiasis is an illness that occurs frequently in small ruminants that are fed grain-rich diets. It is highly lethal, despite being responsive to clinical and surgical treatment (Riet-Correa *et al.*, 2008). Over the last few decades there has been a considerable increase in the number of cases of obstructive urolithiasis in small ruminants, especially in sheep (Ortolani, 1996). Urolith formation has been correlated with intake of high concentrations of energy-rich grains for a prolonged period. Many of these grains have a low Ca/P ratio (Emeric and Embry, 1964; Godwins and Williams, 1982).

In addition to the nutritional component, there is also a factor that boosts urolith formation based on feeding behaviour. In ruminants, there is very little excretion of phosphorus in the urine. Their serum phosphorus levels are due both to the amount of phosphorus obtained from the feed and to the amount in the saliva, which recycles this macroelement and thus participates in metabolising the rumen microbiota. If the amount of saliva produced and secreted decreases, much of the phosphorus starts to be eliminated by the kidneys. Therefore, when there is a high phosphorus level and the pH of the fluid in the tubes is not extremely low, phosphorus can become insoluble and crystallise into stones (Hoar *et al.*, 1969).

Ruminants salivate throughout the day and adult sheep can secrete up to 30L/d of saliva (Bartley, 1976). However, saliva is secreted in different amounts depending on the activity being performed by the animal. Studies on sheep fitted with cannulas in the parotid glands (which are responsible for 47% of total saliva secretion) have shown that about 40% of salivation occurs during rumination, 23% during feed intake and around 37% during resting periods (Bartley, 1976). Small ruminants normally dedicate 6 to 8 h/d to rumination, reaching a maximum of 10 h/d. Although the time spent ruminating is less than the time spent resting, the saliva production during rumination is close to two and half times greater than what is observed during resting. Time spent ruminating is positively influenced by the amount of raw fibre in the diet (Stone, 2004).

Feed concentrates, especially grains, are very rich in energy, proteins and phosphorus (Bartley, 1976), but are also low in effective raw fibre and calcium, which predisposes animals towards the emergence of urolithiasis. When a diet is predominantly composed of grains, there is a drastic reduction in the time spent ruminating (close to four times shorter than when a forage diet is provided), thereby decreasing salivation. If maize is simply broken or is ground up, this reduces the effectiveness of the fibre (respectively, reduction by 40% and 52%) (Russell *et al.*, 1992). A study on sheep showed that both pelleting and grinding of grains (Bartley, 1976) also negatively interfered with salivation, thus drastically increasing the incidence of urolithiasis (Crookshank *et al.*, 1967).

With the recent intensification of small ruminant farming, the percentage of energy-rich concentrates in the diet has increased in order to increase productivity and weight gain and to reduce the finishing period for lambs and goats (Papia *et al.*, 2011; Pompeu *et al.*, 2009). One alternative for replacing traditional energy-rich grains is citrus pulp, a by-product from the orange juice industry. This is treated with limestone, dried and pelleted. In addition to being an energy and calcium-rich food, this pulp is low in phosphorus and magnesium, and has a relatively high effective raw fibre content of 17.5%, compared to the 4.32% of cornmeal (Russell *et al.*, 1992, Bampidis and Robinson, 2006; Barrêto-Júnior *et al.*, 2008). Studies on dairy cows have shown that this concentrate can replace part of the maize and has advantages, since it is cheaper, reducing the risk that rumen

acidosis might appear and stimulate rumination (Carvalho, 1995).

The majority of studies that have evaluated the effectiveness of citrus pulp for stimulating rumination and indirectly stimulating salivation were conducted on dairy cows (Stone, 2004). Therefore, it is necessary to develop tests that estimate these results among sheep. Furthermore, the capacity of stimulate rumination of the citrus pulp meal and the pelleted pulp (normally available in the domestic market) in sheep has not yet been evaluated comparatively. Another point that has not been studied is comparison of these two forms of citrus pulp with other traditionally used grains (maize, wheat and rice), compared to the time devoted to rumination in this species.

In the present study our aim was to examine the effects of different feed concentrates on sheep behaviour. Our hypothesis was that that citrus pulp would stimulate rumination, reducing the risk of urolithiasis, and thus be capable of replacing other concentrates traditionally used for feeding sheep in confinement, especially in farms with high incidence of this disease.

MATERIALS AND METHODS

The study used ten healthy adult (3-4 years) male crossbred Santa Inês sheep that had been castrated. They were kept in individual metabolism cages throughout the experimental period. One month before the start of the experiment the animals were dewormed and habituated to the experimental conditions, receiving a baseline diet containing 80% chopped hay produced from coast-cross grass and 20% commercial concentrate feed (Fri-Ovino 22/70, Nutreco Nutrição Animal, São Paulo, Brazil), to provide a weight gain of 100g/d. The animals had a previous forage-base diet, with no concentrate supplementation, since they are from extensive-system farms.

A Latin square experimental design was used to evaluate five different diets. Four diets were formulated as 30% dry matter intake from coast-cross hay plus 70% concentrate made from pelleted citrus pulp, citrus pulp meal, cornmeal or wheat bran, and one diet, considered the control diet, had similar composition to the basal diet (80% hay and 20 commercial concentrate). Table 1 presents the chemical composition of the hay and the concentrates used.

Table 1. Chemical composition of the hay and the concentrate used during the experiment.

Composition	Coast-cross hay	Concentrate	Citrus pulp	Wheat bran	Corn meal
Dry mater	86.8	87.0	85,3	90,1	89,2
NDF*	32,4	16,0	25,15	45,6	15,5
Crude protein	7,8	14,0	7,12	17,2	8,8
Ether extract	1,8	2,0	1,68	2,4	4,1
Ash	6,3	16,0	5,4	4,5	3,2

*Neutral detergent fiber

Each stage of the Latin square lasted 21d, and on the last day the behavioural observation was made. To avoid the risk of urolithiasis development due to continuous intake of consecutive calculogenic diets, the animals were fed with the baseline diet over a 7 day interregnum between the stages, and then were fed the planned feed. To avoid the risk of rumen lactic acidosis, the following dietary management was used during 21d in each stage: for the first 7d, intake of 40% concentrate and 60% hay; from the 8th to the 14th day, intake of 50% concentrate and 50% hay; and from the 15th day onwards, intake of 70% concentrate and 30% hay.

At the start of the 21st day, 24h of visual monitoring was performed on each animal, conducted by a previously trained team. The lengths of time spent eating, time spent resting and the time spent ruminating were monitored and timed. Time spent eating was defined as biting, chewing and swallowing the hay or concentrate. Every observation session was carried out for 24h starting the morning at the time of diet delivery using a scan-sampling technique. Eating, ruminating and resting activities were recorded every 3min, and each behaviour was assumed to persist for the entire 3-min interval. The absolute length of time that the animals spent on each activity was calculated by multiplying the frequency of observations of

each behavioural activity by the 3-min interval (Cozzi and Gottardo, 2005; Lin *et al.*, 2011).

A circadian evaluation of the results from the observations was also conducted, in which the daytime period was taken to be from 06:00h to 18:00h, and the night-time period was taken to be from 18:00h to 06:00h. This was used as another variable for inclusion in the calculations of the results, thereby enabling circadian evaluation of sheep behaviour. During the observations, we also took into account the animals' positions, noting whether they were standing or lying down.

During the night, weak artificial illumination was used, with the sole purpose of making it possible to see the sheep. The observer team ensured that minimal noise was made, to avoid interference with the nocturnal behaviour of the sheep. The same light was used throughout the experiment.

The visual monitoring data were used to estimate the total amount of saliva produced, in accordance with information from Beauchemin (1991), who reported the production of 25, 18 and 8 mL/min during rumination, feed intake and resting, respectively.

All the statistical analyses were conducted using the Minitab statistical software. To compare the variables, all data were tested for normal distribution using the Kolmogorov-Smirnov test. All the results presented normal distribution. Initially, a descriptive analysis was performed to obtain the total amount of time that each animal spent on resting, intake and rumination and the coefficients of variation. Analysis of variance was chosen for comparisons between the five different experimental groups (diets), followed by Tukey's post-hoc test, using a 5% significance level. The circadian assessment (day versus night) and animal position (standing versus lying down) were evaluated separately for each group using Two-sample T Test. Regression analyses were performed between pairs of variables.

RESULTS

Table 2 shows a comparison between the experimental groups for each of the functional activities observed (time spent eating, time spent resting and time spent ruminating) and the estimated saliva production. This table indicates that pelleted citrus pulp and citrus pulp meal diets resulted in amounts of time spent on rumination that were similar to the findings from the control diet, but greater than for cornmeal.

Table 2. Total time (mean±SD) and percentage of rumination, food intake and sheep rest during 24 hours of behavior observation

Treatment	Time spent ruminating				Time spent eating				time spent resting			
	min (M ± SD)		(%)	CV	Min (M ± SD)		(%)	CV	min (M ± SD)		(%)	CV
Control diet	668.8a	96.5	46.4	14.4	346.2a	77.7	24.0	22.4	427.5c	103.9	29.6	24.3
Pelleted citrus pulp	601.1ab	34.2	41.7	5.7	234.0b	30.3	16.3	12.9	604.9b	57.8	42.0	9.6
Citrus pulp meal	589.9ab	53.4	40.9	9.1	228.7b	30.7	15.9	13.4	622.9b	84.3	43.2	13.5
Wheat bran	483.0bc	87.5	33.6	18.1	229.9b	52.1	15.9	22.6	726.7ab	114.5	50.5	15.8
Corn meal	421.5c	125.3	29.3	29.7	199.9b	66.8	13.9	33.4	818.6a	119.5	56.8	14.4

Different letters in same column indicate difference throughout ANOVA and Tukey test (P<0.05).

As expected in the hypothesis of this study, effective rumination was shown to be generated by the citrus pulp feed. However, it was expected that the citrus pulp meal could cause shorter rumination than would be seen with the pelleted citrus pulp. It is possible that this did not occur because the grinding of the granules did not reduce the particles completely, thereby leading to a requirement for normal durations of rumination. The longer time spent ruminating that had been expected in the control diet, in comparison with the cornmeal and wheat bran groups, was confirmed. Contrary to expectations, the groups fed with pulp did not differ from the wheat bran group, perhaps due to the high standard deviations of these groups.

The influence of the animal factor on the time spent ruminating was also analysed, taking into account all the diets studied, and no significant differences were seen (P = 0.658). Nevertheless, the mean difference between the animals with

highest and lowest time spent ruminating reached 22%.

When the sheep were consuming the control diet, they had a higher time spent eating. Hay consumption, which was greatest in the control diet, was slower and better distributed throughout the day than the intake of concentrates.

The control diet resulted in a shorter time spent resting than observed with the remaining diets. This was expected, since more time was spent eating in the control diet and since this diet also stimulated rumination, thus reducing time spent resting. Coincidently, the longer the time spent ruminating (r^2 = 0.83) and time spent eating (r^2 = 0.53), the shorter the time spent resting (P<0.05). Table 3 presents the estimated saliva production in 24 hours of behavior observation of the different groups. The saliva production in the control diet was greater than in the other groups, and was greater in the groups fed with citric pulp in comparison with the cornmeal group.

Table 4 shows a comparison between the experimental groups regarding the time spent ruminating, time spent eating and time spent resting in relation to daytime and night-time. The control diet presented longer time spent ruminating during the night. Conversely, all the other groups presented opposite results, with higher time spent ruminating during the day. The control diet presented equal distribution of time spent resting between daytime and night-time, while all the other groups had more time spent resting at night. No differences in time spent eating were observed among the experimental groups, with the exception of the control diet, which had greater time spent eating during daytime.

Table 3. Estimated saliva production in 24 hours of behavior observation of sheep in different treatments

Treatment	Estimated saliva production (L)	
	Mean	SD
Control diet	26.4a	1.7
Pelleted citrus pulp	24.1b	0.7
Citrus pulp meal	23.8b	1.1
Wheat bran	22.0bc	1.7
Corn meal	20.7c	1.9

Different letters in same column indicate difference throughout ANOVA and Tukey test (P < 0.05).

Table 4. Data of time spent ruminating, time spent eating and time spent resting in minutes (mean±SD) and percentage, considering the diurnal and nocturne periods during the observation period

Treatment	Day			Night			P*
	Minutes (mean ± SD)		%	Minutes (mean ± SD)		%	
Time spent ruminating							
Control diet	298.1	38.5	44.6	370.6	71.9	55.4	0.031
Pelleted citrus pulp	355.1	51.9	59.1	246.0	48.2	40.9	0.001
Citrus pulp meal	333.8	22.0	56.6	256.1	52.7	43.4	0.004
Wheat bran	285.0	44.6	59.0	198.0	73.5	41.0	0.015
Corn meal	244.9	88.0	58.1	176.6	48.4	41.9	0.049
Time spent resting							
Control diet	217.5	57.8	50.9	210.0	68.3	49.1	0.816
Pelleted citrus pulp	245.6	52.1	40.6	359.3	57.6	59.4	0.001
Citrus pulp meal	267.0	30.4	42.9	355.9	69.2	57.1	0.009
Wheat bran	322.5	59.7	44.4	404.3	82.8	55.6	0.043
Corn meal	369.8	90.2	45.2	448.9	47.5	54.8	0.050
Time spent eating							
Control diet	204.4	54.5	59.0	141.9	30.0	41.0	0.017
Pelleted citrus pulp	119.3	15.9	51.0	114.8	16.4	49.0	0.587
Citrus pulp meal	118.5	18.4	51.8	110.3	22.5	48.2	0.437
Wheat bran	112.5	31.5	48.9	117.4	28.6	51.1	0.751
Corn meal	105.4	39.5	52.7	94.5	31.2	47.3	0.551

*Statistical analysis throughout T test in each treatment separately to compare day vs. night periods.

Regarding the animals' position, all sheep fed in a standing position. For resting, the control diet and cornmeal groups rested more in a lying down position (73.4 and 64.3% respectively; P<0.05), while none of the other groups presented any differences in positions of resting, with 53.7, 58.4 and 52.0% of the resting time on lying down position for the groups Pelleted citrus pulp, Citrus pulp meal and Wheat bran, respectively. All the experimental groups presented greater time spent ruminating(P<0.05) in lying down position, with 96.6, 91.9, 90.9, 84.5 and 88.1% for the Control diet, Pelleted citrus pulp, Citrus pulp meal, Wheat bran and Corn meal groups, respectively.

DISCUSSION

When the sheep were consuming the control diet, they spent a longer time eating. The consumption of forage was more uniform and much better distributed throughout the day than the consumption of concentrates, thus confirming the description by Beauchemin (1991).

The time spent ruminating in the experimental groups showed coefficients of variance that tended to be greater than what was described by Beauchemin and Buchanan-Smith (1989) for dairy cattle, which was around 10%. This may be explained by the longer time spent ruminating that sheep exhibit, compared with cattle, thereby favouring greater dispersal of the results and, consequently, a higher coefficient of variation for behaviour among animals consuming a diet similar to that of dairy cattle (Mendes Neto *et al.*, 2007).

The diets that contained citrus pulp resulted in more time spent eating and time spent ruminating. These were greater than with the cornmeal and wheat bran diets, and consequently the time spent resting was shorter than with the other treatments. As suggested in our hypothesis, both the pelleted citrus pulp and the citrus pulp meal significantly stimulated rumination, with time spent in rumination similar to what was observed in the control diet but longer than with the cornmeal group.

Although the pelleted citrus pulp and citrus pulp meal had lower NDF than the wheat bran, the two citrus pulp concentrates had higher percentages of particles of size greater than one millimetre, which also stimulated rumination. Even after going through a grinding process, the citrus pulp did not show any significant reduction in its capacity to stimulate rumination, which was because the final sizes of the particles were still large enough to stimulate the mechanoreceptors in the rumen wall. These data differ from what was observed by Bartley (1976), who found in a study on grain-fed sheep that the pelleting and grinding effects interfered negatively with saliva production. Probably the high amount of fiber in the citric pulp had a major impact on time spent ruminating.

When the sheep were fed cornmeal, the time spent resting was higher than when fed with either of the citrus pulp concentrates, probably because cornmeal promoted shorter time spent ruminating. The concentrate-rich diets led the animals to spend time ruminating more during the day than at night, while the inverse was observed with the control diet (P<0.05). The time spent eating was identical during the day and night with the concentrate-rich diets, while it was greater during the day with the control diet (P<0.05). When the sheep were fed concentrate-rich diets, the time spent resting was higher during the night (P<0.05). With all the diets, around 90% of the time spent ruminating occurred with the animals lying down.

When sheep receive concentrate-rich diets, they spent longer periods ruminating during the day, regardless of the experimental group, and spent time resting more during the night, thus showing a tendency to spend time eating during the day. These data differ from what was found for cattle (Mendes Neto *et al.*, 2007) that were fed with different percentages of citrus pulp. Analysis of the rumination data according to the diet provided showed that the concentrate-rich diets led the sheep to ruminate more during the day, in a similar way to what has been observed among cattle and goats (Beauchemin, 1991; Desnoyers *et al.*, 2009), and inversely to what has been observed among grazing sheep, which mainly ruminate and rest during darkness (Lin *et al.* 2011). Despite our experimental conditions, with artificial illumination, the control diet presented behaviour similar to grazing sheep and spent more time ruminating during the night time.

Considering that saliva is the main metabolic pathway for phosphorus excretion (Hoar *et al.*,

1969), the greater the production of saliva is, the greater the amount of phosphorus excreted to the rumen content will be, and consequently, the lower the urinary excretion of phosphorus. This reduces the risk for urolithiasis in sheep. Hence, it was observed that the diets with large amounts of pelleted citrus pulp and citrus pulp meal resulted in lower predisposition towards urolithiasis than diets with large amounts of cornmeal.

CONCLUSIONS

Citrus pulp, both in pellet and meal form, and wheat bran to a lesser degree, adequately promoted rumination; the milling process on the citrus pulp did not interfere with the time spent ruminating in sheep. Concentrate-rich diets favoured time spent ruminating during the day and time spent resting during the night. As expected in the hypothesis of this study, the citrus pulp induced higher effective rumination. The use of this concentrate can act as a preventive measure to reduce the incidence of urolithiasis in sheep flocks.

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