

Mammary artery Doppler ultrasonography of Brazilian Bergamasca dairy ewe lambs under the influence of two different feeding plans¹

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ABSTRACT.- Dantas A., Siqueira E.R., Fernandes S., Oba E., Machado V.M.V., Castilho A.M., Sartori M.M.P. & Santos R.V. 2017. **Mammary artery Doppler ultrasonography of Brazilian Bergamasca dairy ewe lambs under the influence of two different feeding plans.** *Pesquisa Veterinária Brasileira* 37(2):179-182. Departamento de Produção Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Rua José Barbosa de Barros 1780, Botucatu, SP 18618-000, Brazil. E-mail: dantas.vet@gmail.com

Blood supply of mammary gland is extremely important, since it is a crucial factor in the mammary tissue development. The objective of this study was to describe and assess main mammary artery hemodynamic characteristics of lambs with two growth rates, using Doppler ultrasound. Two experimental groups, A and B, (n=10) of female Brazilian Bergamasca lambs were kept under the same management conditions and facilities. There were two phases: Phase 1, from 90 days of age until onset of puberty; Phase 2, from puberty until 1 year of age. During phase 1, a specific diet was formulated to each experimental group to obtain two different average daily gains: 150g, for treatment A, and 250g, for treatment B. During phase 2, there was no difference on the diet offered to both groups. Every 14 days, all animals were weighed and their body condition score was evaluated. Every 60 days, ultrasound exams (Doppler) of mammary artery were performed to obtain resistance and pulsatility indexes. Treatment B lambs showed higher weight gain and higher body condition score (P<0.05). However, for resistance and pulsatility indexes, there was no difference between treatments (P>0.05). Hemodynamic values remained constant, independent of diet or body development presented by the animals. Obtained data is valuable information on Bergamasca ewe lambs development.

INDEX TERMS: Doppler, ultrasonography, ultrasound, mammary artery, dairy ewes, Brazilian Bergamasca, feeding plans, blood flow, dairy lamb, nutrition.

RESUMO.- [Avaliação ultrassonográfica por Doppler da artéria mamária de cordeiras da raça Bergamácia Brasileira sob a influência de dois planos de alimentação

diferentes.] O suprimento sanguíneo da glândula mamária é extremamente importante, uma vez que é um fator crucial para o desenvolvimento do tecido mamário. Assim, a necessidade de registro dos parâmetros de fluxo sanguíneo arterial é evidente. O objetivo do estudo foi descrever e avaliar as principais características hemodinâmicas da artéria mamária de cordeiras submetidas a diferentes taxas de crescimento, usando o ultrassom Doppler. Dois grupos experimentais, A e B, (n=10) de cordeiras da raça Bergamácia brasileira foram mantidas sob as mesmas condições de manejo e instalações. O estudo foi dividido em diferentes fases, sendo: Fase 1, de 90 dias de idade até o início da puberdade; Fase 2, desde a puberdade até 1 ano de idade. Durante a fase 1, dietas específicas foram formuladas para cada grupo experimental, a fim de obter duas médias dife-

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rentes de ganho de peso diário: 150g, para o tratamento A, e 250g, para o tratamento B. Durante a Fase 2, não houve diferença na dieta oferecida para os dois grupos. A cada 14 dias, todos os animais foram pesados e determinado o escore de condição corporal. A cada 60 dias, exames de ultrassom (Doppler) da artéria mamária foram realizados a fim de calcular os índices de resistência e pulsatilidade. As cordeiras do tratamento B registrou maior ganho de peso e escore de condição corporal ($P < 0,05$). No entanto, não houve diferença para os índices de resistência e pulsatilidade entre os tratamentos ($P > 0,05$). Os valores hemodinâmicos permaneceram constantes, independente da dieta ou o desenvolvimento do corpo apresentada pelos animais. Assim, os dados obtidos são informações valiosas sobre o desenvolvimento da raça a que se refere.

TERMOS DE INDEXAÇÃO: Ultrassonográfica, Doppler, artéria mamária, cordeiras, Bergamácia Brasileira, planos de alimentação, fluxo sanguíneo, cordeira leiteira, nutrição.

INTRODUCTION

Mammary gland development starts even before birth and continues parallel to the different stages of reproductive life of the female in postnatal period, which concentrates most of the udder growing. However, nutritional management has a significant impact on mammary gland development.

Malnutrition can affect mammary growth negatively, reducing future milk production (Akers et al. 2000). Thus, the development of a nutritional management to equalize body and udder development is fundamental, especially during growth phase, being of utmost importance to carry out studies in this area.

Ultrasound proved to be a very useful technique to study *in vivo* morphology and internal activity of sheep mammary gland (Piccione et al. 2004, Petridis et al. 2014). Using Doppler, hemodynamic parameters can be measured, allowing the evaluation of metabolic status on various organs. However, little is known about hemodynamic characteristics of sheep mammary artery, especially on Brazilian Bergamasca, which have a considerable dairy potential.

The objective of this study was to describe the found values for resistance index and pulsatility index of left and right mammary arteries of Brazilian Bergamasca dairy ewe lambs with two growth rates and assess whether the referred values vary during this period.

MATERIALS AND METHODS

The Ethics Committee on the Use of Animals (CEUA), Faculty of Veterinary Medicine and Animal Science, UNESP/Botucatu approved the study, registering it on Protocol 159/2010.

Location, animals and experimental treatments. Twenty Bergamasca lambs (simple delivery born) were randomly assigned to two groups ($n=10$). The animals were confined throughout experimental stage, being two animals in the same treatment/per pen.

There were two phases: 1, from 90 days to onset of puberty (prepubertal females were daily exposed to a vasectomized ram and onset of puberty was considered when the female accepted coupling); and 2, from puberty until 1 year of age.

For phase 1, two types of diets were formulated, to obtain average daily gain of about 150g (treatment A) and average daily

gain around 250g (treatment B). In phase 2, a balanced diet was provided to both groups. Diets details are in Table 1.

Diet and general management. Diets, formulated according to the National Research Council (NRC 2007) to meet the requirements of animals throughout experimental period, were offered twice daily (8:00am and 3:00pm) in *ad libitum* feeding regimen and the amount of food was adjusted daily according to the remains (which should be about 10% of the offered on the previous day).

Weighing, body condition score and dry matter intake. Lambs were individually weighed every 14 days on a digital scale. Weight was measured in kilograms (kg) and average daily gain (ADG) expressed in grams (g). During weighing, body condition score (BCS) was checked through palpation and values were given in a scale from 1 to 5 (subunits of 0.25), where 1 represents overly lean animals and 5 those extremely fat (Russell 1984).

Dry matter intake was measured daily, adjusting the provided amount of food according to the remains from the previous day (kg/day).

Sonographic Review. Four sonographic evaluations were performed with an interval of 60 days, all made by the same operator using the same device (Esaote® Mylab model 30). Examinations were performed in non-anesthetized, standing animals.

An 18 MHz microlinear transducer was used, due to its ultrasonic wave magnitude and appropriate image resolution. To improve contact and eliminate air between the wall of the udder and the transducer, carboxymethylcellulose gel was applied.

Transducer was placed directly on caudal surface of mammary gland and parallel to the teat. Ultrasound images were initially obtained from a sagittal plane and, later, axial plane, both in B-MODE. To locate arteries, we used Color Doppler system and, then, quantitative evaluations were made from the initial fraction of left and right mammary arteries, respectively, using Spectral Doppler.

Insonation Angle was adjusted, so ultrasound beam passed crossing the course of blood flow into the vessel, without exceeding 60°. All described evaluations were firstly performed on left mammary artery and repeated on right mammary artery, for every animal.

Obtained images were evaluated based on spectral form of waves outline. Then, resistance index (RI) and pulsatility index (PI) of each vessel was calculated, according to the following formula: $RI = (PSV-EDV)/PSV$ and $PI = (PSV-EDV)/TAMV$, where PSV

Table 1. Formulation and composition of experimental diets

Item	Phase 1 ^b		Phase 2 ^c
	Treatment A	Treatment B	A/B
Coast Cross [®] Hay	62.45	20.20	18.81
Ground corn	23.58	57.23	68.85
Soybean Meal	11.84	20.20	9.87
Urea	-	-	4.80
Ammonium chloride	0.00	0.00	0.05
Limestone	0.61	0.10	0.10
Mineral Salt ^a	1.21	1.04	1.07
Monensin	0.30	0.30	0.30
Dry Matter	90.81	89.55	89.84
Crude Protein	11.34	16.04	14.20
Total Digestible Nutrients	65.12	78.20	79.98
Ether Extract	1.70	3.44	5.16
Mineral Matter	5.46	4.53	4.73
Neutral Detergent Fiber	56.39	28.00	25.91
Acid Detergent Fiber	37.44	17.05	9.63

^a Mineral Salt Composition (kg of product): Ca = 155g, P = 65g, Mg = 6g, S = 12g, Na = 115g, Se = 27mg, Cu = 100mg, Fe = 1000mg, Zn = 6000mg, Mn = 140mg, I = 175mg, Co = 175mg, F 1650mg; ^b 90 days of age to puberty onset, ^c puberty to 1 year old.

Table 2. Means and standard deviations of initial and final weight, average daily gain, average daily dry matter intake and body condition score of lambs of treatment A and B

	Variable	Treatment A	Treatment B
Phase 1 ^a	Initial Weight (kg)	14.95 ± 1.98	13.90 ± 1.53*
	Final Weight (kg)	28.77 ± 3.62	36.95 ± 3.00*
	Average Daily Gain (g)	140.00 ± 0.06	235.00 ± 0.09*
	Average Daily Dry Matter Intake (kg)	0.83 ± 0.14	0.96 ± 0.07
	Body Condition Score (1-5)	1.42 ± 0.51	1.74 ± 0.76*
Phase 2 ^b	Initial Weight (kg)	29.39 ± 3.09	38.55 ± 2.86*
	Final Weight (kg)	57.17 ± 4.09	65.17 ± 3.86*
	Average Daily Gain (g)	174.00 ± 0.07	170.00 ± 0.05
	Average Daily Dry Matter Intake (kg)	1.23 ± 0.08*	1.08 ± 0.08
	Body Condition Score (1-5)	2.76 ± 0.24	3.12 ± 0.22*

^a 90 days of age to puberty onset, ^b puberty to 1 year old, *P<0.05.

Table 3. Means and standard deviations for resistance and pulsatility indexes of left and right mammary gland of lambs under to two distinct treatments

Time points ^a	Resistance Index			
	Treatment A LMG ^b	Treatment B LMG ^b	Treatment A RMG ^c	Treatment B RMG ^c
1	0.69 ± 0.08	0.66 ± 0.09	0.67 ± 0.08	0.68 ± 0.11
2	0.62 ± 0.06	0.63 ± 0.10	0.64 ± 0.06	0.63 ± 0.09
3	0.60 ± 0.11	0.68 ± 0.09	0.63 ± 0.09	0.64 ± 0.10
4	0.65 ± 0.14	0.66 ± 0.10	0.63 ± 0.08	0.63 ± 0.11
	Pulsatility Index			
1	1.82 ± 0.59	1.59 ± 0.64	1.68 ± 0.54	1.70 ± 0.57
2	1.16 ± 0.23	1.24 ± 0.42	1.27 ± 0.37	1.27 ± 0.37
3	1.15 ± 0.36	1.56 ± 0.45	1.27 ± 0.40	1.40 ± 0.47
4	1.41 ± 0.56	1.57 ± 0.55	1.30 ± 0.43	1.35 ± 0.54

^a Every 60 days, ^b left mammary gland, ^c right mammary gland, P>0.05.

is peak systolic velocity, EDV is end diastolic velocity and TAMV is time-averaged maximum velocity (Ginther 2007).

Statistical analysis. The experiment was conducted in a completely randomized design and we used a split-plot analysis for resistance and pulsatility indexes, considering as sub-plots: the two growth rates, the main plots and the evaluation period of each analyzed variable. Statistical analysis was performed using statistical software Minitab 16 (Minitab 2010).

RESULTS

Weight, average daily gain, daily dry matter intake and body condition score

Variables related to body development were influenced by diet plan because lambs in treatment B showed better results than lambs in treatment A, during phases 1 and 2 (P<0.05).

Resistance and pulsatility indexes

Evaluated parameters (RI, PI) showed constant values during the development of mammary gland, with no differences between right and left sides, for treatments and for analyzed time points (P>0.05).

DISCUSSION

The analyzed parameters for body development reflected the expected differences between the two diets (Table 1).

Treatment B lambs underwent to a high nutritional level and energy concentration, providing increased availability of nutrients to body growth and allowing superior weight gain during phase 1 (Medeiros et al. 2007, Jacques et al. 2011).

During phase 2, growth rate on treatment B lambs showed a gradual slowdown, probably due to proximity of expected weight at maturity. However, treatment A lambs had an increase on growth performance, possibly, because of diet changes and less need for maintenance of these animals during phase 2, noting up a better diet use during the reimplantation period.

In Doppler evaluation, changes in the PI and RI values (Table 3) were not observed, indicating regular and constant blood perfusion, without vasoconstriction or vasodilation, suggesting the development of a normal blood requirement autoregulation through the tissue, providing the growing mammary gland needs (since both treatments showed satisfactory mammary development). In this regard, it is noteworthy to say that, in these sonographic evaluations, there were not abnormal changes in any of mammary glands analyzed during the trial period.

RI values found in this study also suggest a moderate metabolism of mammary tissue, which is compatible with the mammary gland development phase on which the examined lambs were. In ruminant, mammary development during prepubertal period is high; however, it occurs sparingly, compared to development on the end of pregnancy, where it reaches its maximum rates (Sejrsen & Purup 1997). Anderson (1975) showed that, in Romney Marsh sheep, 20% of mammary gland growth occurred between birth and puberty, 78% during pregnancy, and 2% during lactation.

The PI and RI data of this study, as observed by Petridis et al. (2014) evaluating sonographic findings of sheep mammary gland during mammary involution period, indicate that blood requirement of mammary tissue was moderate. This suggests absence of overload in circulatory system and satisfactory blood distribution among other tissues, favoring body development, which is intense during growth phase.

In view of this, the importance of proper attention to a precise nutritional management of lambs during growth phase is reinforced. Although distinct diets and average weight gain resulted in different growth rates, it was not sufficient to interfere on hemodynamic parameters of lamb mammary arteries on both treatments. Thus, the use of a more intensive feeding plan during pre-pubertal stage, in order to speed up mammary development, is not reasonable.

CONCLUSIONS

Indexes related to Doppler ultrasonography of lambs left and right mammary artery, on both treatments, remained constant throughout growth phase and were similar, regardless of the nutritional management used.

These results, when combined with performance related factors, can be used as guidelines for interpreting hemodynamic characteristics of dairy potential lambs.

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