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# Influence of feeding differentiation on the age at onset of puberty in Brazilian Bergamasca dairy ewe lambs

[*Influência da diferenciação alimentar na idade à puberdade em cordeiras da raça Bergamácia Brasileira*]

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## ABSTRACT

The objective of the study was to evaluate body growth and age at onset of puberty on lambs fed two specific diets for low and high growth rates. A herd of 20 Brazilian Bergamasca lambs was divided in two groups (n= 10) and kept confined throughout the experimental period, two animals of the same treatment/pen. Two phases were established: Phase 1, from 90 days of age until the onset of puberty; and Phase 2, from puberty onset up to 1 year old. For Phase 1, two distinct diets were formulated, being: Treatment A, which was formulated to obtain an average daily gain of approximately 150g; and Treatment B, for an average daily gain of about 250g. In Phase 2, a balanced, equal diet was provided to both groups. Every 14 days, the animals were weighed and given average daily gain, average daily dry matter intake and body condition score. From the 5$^{th}$ month of age on, in each group, a vasectomized male was used to detect estrus, establishing age at puberty onset and estrus interval for each lamb. Blood samples were collected every 28 days to determine plasma growth hormone concentration. Treatment B lambs gained more weight and had higher body condition score ($P<0.05$) and there was no difference for age at puberty onset and plasma growth hormone levels ($P>0.05$) between treatments. It was found that both treatments showed satisfactory performances. Thus, treatment A may be indicated as a reasonable feeding system to achieve positive responses on confined ewe lambs during growth phase.

Keywords: nutrition, performance, reproduction, growth hormone, sheep

## RESUMO

*O objetivo do estudo foi avaliar o crescimento corporal e idade de início da puberdade em cordeiras sob duas dietas específicas para taxas de crescimento baixas e altas. Um rebanho de 20 cordeiras Bergamácia Brasileira foi dividido em dois grupos (n= 10) e mantido confinado em todo período experimental, sendo dispostos dois animais do mesmo tratamento/baia. Duas fases foram estabelecidas: Fase 1, a partir de 90 dias de idade até o início da puberdade, e Fase 2, a partir do início da puberdade até 1 ano de idade. Para a fase 1, duas dietas distintas foram formuladas, sendo: Tratamento A, formulada para obter um ganho de peso diário de aproximadamente 150g; e Tratamento B, para um ganho de peso médio diário de cerca de 250g. Na Fase 2, uma dieta equilibrada idêntica foi fornecida para ambos os grupos. A cada 14 dias, os animais foram pesados e calcularam-se o ganho médio diário, o consumo médio diário de matéria seca e escore de condição corporal. A partir do quinto mês de idade, em cada grupo, um macho vasectomizado foi usado para detectar estro, estabelecendo a idade de início da puberdade e estro para cada cordeira. As amostras de sangue foram coletadas a cada 28 dias para determinar a concentração plasmática de hormônio do crescimento. Cordeiras do tratamento B ganharam mais peso e tiveram maior escore de condição corporal ($P<0,05$), mas não houve diferença de idade para o início da puberdade e para os níveis plasmáticos de hormônio do crescimento ($P>0,05$). Verificou-se que os tratamentos A e B apresentaram desempenhos satisfatórios. Assim, o tratamento A pode ser indicado como um sistema de alimentação para alcançar respostas positivas em cordeiras submetidas ao confinamento durante a fase de crescimento.*

*Palavras-chave: nutrição, desempenho, reprodução, hormônio do crescimento, ovino*

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## INTRODUCTION

Several studies have shown that offering a high nutritional plan not only provides a high rate of weight gain and body condition score, but also results in a lower age at onset of puberty (Rosales Nieto, *et al.*, 2011, 2013) which emphasizes the importance of adequate nutrition.

Growth hormone (GH) is an essential component of animal physiology regulation, metabolism and growth. Its plasma concentration is related to the nutritional management that is offered. In general, animals subjected to a low dietary plan have elevated plasma GH levels (Thomas *et al.*, 1990) which results in mobilization of adipose tissue. Therefore, appropriate dietary supply of resources is required for achieving increased growth and full expression of genetic capabilities.

Considering the Brazilian Bergamasca dairy potential, conducting research to provide alternatives for production of standard quality animals (concerning body development during growth stage) is interesting to dairy farming. It enables to estimate more accurately what kind of food plan is necessary to achieve the best production results in replacement ewe lambs.

The objective of the study was to evaluate the results of two growth rates in ewe lambs during Phase 1 (from 90 days of age until the onset of puberty) and the results of this variation on Phase 2 (from puberty onset up to 1 year old) by monitoring standard performance indexes.

## MATERIAL AND METHODS

The experiment was conducted from July 2011 to April 2012 at School of Veterinary Medicine and Animal Science, in Botucatu, São Paulo, Brazil (42º 52' S, 48º 25' W), whose Ethics Committee approved the experimental design (Protocol number: 159/2010).

We used 20 Brazilian Bergamasca lambs coming from simple deliveries that were randomly assigned to two groups (n= 10). Animals were confined throughout the experimental stage in 3.50m x 1.75m indoor pens (concrete floor, 0.95m x 0.35m feeding through and an automatic drinking fountain) with two animals of the same treatment per pen.

Two phases were established: Phase 1 (90 days of age until the onset of puberty) and Phase 2 (puberty up to 1 year old). For Phase 1, two diets were formulated: A to a 150g average daily gain (ADG); B to a 250g ADG. For Phase 2, a balanced diet was provided to both groups. Diets details are in Table 1. Diets were formulated according to National Research Council (2007).

Table 1. Formulation and composition of experimental diets

| Item | Phase 1[2] | | Phase 2[3] |
|---|---|---|---|
| | A | 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[1] | 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 |

[1]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
[2]90 days of age until the onset of puberty
[3]puberty to 1 year old

Animals were fed twice a day, at 8:00 am and 3:00 pm, with *ad libitum* access to food and water. The amount supplied was daily adjusted according to the remains, which should be 10% of the amount provided on the preceding day.

Dry matter (DM), crude protein (CP), total digestible nutrients (TDN), ether extract (EE), mineral matter (MM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of forage and concentrate feed samples were analyzed at Laboratory of Food Science, Department of Nutrition and Animal Breeding FMVZ-UNESP, Botucatu.

DM, CP, EE and MM were analyzed according to methodology described by Silva and Queiroz (2002). Sequential analysis method proposed by Van Soest (1991) was used to determine NDF and ADF fractions. TDN was calculated as National Research Council (2001).

Every 14 days, lambs were individually weighed on a digital scale. Weight was measured in kilograms (kg) and average daily gain expressed in grams (g).

During weighing, body condition score (BCS) was determined by palpation of the transverse apophysis, giving values in a scale from 1 to 5 (with allocation of 0.25 fractional notes), where 1 represents overly lean animals and 5 those extremely fat (Sañudo and Sierra, 1986).

Average daily dry matter intake (DMI) was calculated for each individual (kg/day) and was determined by subtracting the daily leftover from the offered amount throughout the experimental period.

Individual natural estrus detection was carried out on ewe lambs from 5 months of ageusing a vasectomized ram, which was allocated in the same pen, once daily and in both groups. Age at onset of puberty (days) was considered to be when ewe lambs accepted coupling. Estrus interval was defined as the period (days) between observed estruses, during experimental period. Weight at puberty onset (kg) was the weight observed at detection of first estrus.

Blood samples were collected from the jugular vein every 28 days in order to determine GH plasma concentrations at Endocrinology Laboratory of FMVZ-UNESP, Botucatu. The blood was collected into heparinized vacuum tubes and centrifuged at 700 G for 15 minutes to obtain plasma.

Plasma samples were transferred to 2mL identified and dated polyethylene microtubes. Then, these wells were frozen at -20°C until analysis. Plasma GH concentration was determined in duplicate by radioimmunoassay, considering as reference 0.68ng/mL (Wankowska *et al.*, 2008).

The experiment was conducted in a completely randomized design and the variables: weight, ADG, DMI, BCS and age at onset of puberty submitted to analysis of variance. We used split plot analysis for GH evaluation, considering the two growth rates as the main plots and the evaluation period of the variable analyzed as sub-plot. Statistical analysis was performed using a statistical software (Minitab, 2010).

## RESULTS

In Phase 1, differences for weight and ADG were observed between treatments ($P<0.05$). Lambs subjected to treatment B had better performance throughout the experimental period. In Phase 2, for ADG, there was no difference between the two treatments ($P>0.05$).

The evolution of BCS presented ascending pattern (Tab. 2), with a difference between the two treatments during both phases. Best values for treatment B.

In Table 3, age at onset of puberty and the interval between estruses were not different in both treatments ($P>0.05$); however, average weight of Treatment B was higher at puberty onset ($P<0.05$).

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

| | Variable | Treatment A | Treatment B | |
|---|---|---|---|---|
| Phase 1[1] | 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[2] | 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 | * |

[1]90 days of age until the onset of puberty
[2]Puberty to 1 year old
*P<0.05

Table 3. Means and standard deviations for age at onset of puberty, average interval between estruses and weight at onset of puberty of Bergamasca lambs subjected to treatment A and B

| Variable | Treatment A | Treatment B | |
|---|---|---|---|
| Age at onset of puberty (days) | 218.10 ± 15.68 | 215.30 ± 15.12 | |
| Estrus interval (days) | 19.10 ± 4.04 | 18.60 ± 4.35 | |
| Weight at onset of puberty (kg) | 32.98 ± 3.73 | 41.19 ± 4.22 | * |

*P<0.05

There was no difference between phases and treatments (Tab. 4) for the plasma GH concentration (P>0.05), initially presenting as high, decreasing thereafter and remaining low until the end of the experiment.

Table 4. Means and standard deviations of plasmatic concentration of growth hormone (ng/mL) in Bergamasca lambs subjected to treatments A and B

| Moment[1] | Treatment A | Treatment B |
|---|---|---|
| 1 | 3.94 ± 1.93 | 2.64 ± 1.82 |
| 2 | 3.48 ± 1.66 | 1.91 ± 0.88 |
| 3 | 2.42 ± 1.33 | 1.88 ± 0.66 |
| 4 | 1.76 ± 0.26 | 2.16 ± 1.03 |
| 5 | 1.38 ± 0.19 | 1.94 ± 0.56 |
| 6 | 1.38 ± 0.28 | 1.89 ± 1.06 |
| 7 | 1.46 ± 0.25 | 1.58 ± 0.24 |
| 8 | 1.31 ± 0.16 | 1.88 ± 0.41 |

[1]every 28 days
P>0.05

## DISCUSSION

A high proportion concentrate feed/forage has a higher energy content and provides higher availability of nutrients to lamb body growing (Medeiros *et al.*, 2007; Jacques *et al.*, 2011). Treatment B had a higher body weight and higher BCS values (Tab. 2). Frutos *et al.* (1997); Caldeira *et al.* (2007) stated that there is positive relationship between the variables body weight and BCS, reflecting nutritional status.

However, there was no difference for DMI between treatments. Thus, the energy requirement of treatment B lambs may have been achieved with lower levels of dietary intake (Mertens, 1994).

Treatment A showed less weight gain. Probably, the diet offered during phase 1 limited ADG, restricting the growth of the animals, since forages exhibit low nutrients concentration per mass unit and the rate of degradation and escape is slower, restricting food intake (Jung and Allen, 1995). According to Carvalho *et al.* (2007), an increase in the forage:concentrate ratio promotes physical regulation of nutrient intake and, consequently, lower performance in lambs.

For phase 2, treatment B showed higher average body weight and BCS. However, growth rate of treatment B lambs showed a gradual slowdown, probably due to the low ADG (170g), combined with the proximity of expected weight at maturity.

In sheep, growth rate is more intense since birth until reach half of adult weight, decelerating near puberty and, then, gradually decreasing until they reach physiological maturity. Therefore, tham closer the animal is to adulthood, the lower is the increase of body weight in relation to food consumed (Thompson and Parks, 1983). Thus, in treatment B, body development, despite of being higher than A, presented gradual reduction of growth rate, indicating the time of growth stabilization.

The diet offered in phase 2 to treatment A was a stimulus to modify DMI and BCS, resulting in increased weight gain, confirming, thus, better food use by lambs in realimentation stage, as observed by Kamalzadeh *et al.* (1997); Homem Jr. *et al.* (2007). This may be due to less need for maintenance of physiological functions at this stage.

Table 3 shows that the nutritional management imposed to lambs on both treatments, throughout growth phase, led to the onset of puberty at the same age (7 months), confirming Senger (2005) in the variable range between 4 and 14 months. These results suggest that the low-density nutritional diet offered during Phase 1 had no negative effect on age at onset of puberty.

However, it was observed that Treatment A lambs had lower body weight than B (36.74 vs. 44.21kg, respectively), suggesting that, during the entire growth phase, not only diet composition is important but also weight gain rate. According to Gregory *et al.* (1991), age at onset of puberty is mainly a consequence of weight gain rate, therefore, despite the different growth rate of treatment A lambs during Phase 1, they had sufficient time, during Phase 2, to achieve the ideal weight for reproduction (at 218.10 days old).

Several studies show that it is necessary to provide a high nutritional plan for the lambs may reach puberty earlier (Rosales Nieto, *et al.*, 2011, 2013), however, this study showed that it is also possible to achieve this goal in a timely manner with a moderate nutritional plan. According to Wiltbank *et al.* (1966), body weight is only one of the limiting factors in determining the age at onset of puberty in ruminants. After a certain critical level, variation in weight gain has little or no effect on age at onset of puberty (because when animals are subjected to an adequate diet, differences on weight gain are not very relevant).

Lambs of both treatments showed the same estrus interval (19.10 and 18.60 days, respectively) and the same length of estrous cycle, which was within the interval reported by Hafez and Hafez (2004), 17-21 days. Thus, lambs of the two treatments were physiologically ready for reproduction.

As shown in Table 4, plasma GH concentration in both treatments was high during phase 1, gradually reducing along phase 2 and remained low until the end of experiment. Plasma GH levels decline with increasing age and this inverse relationship is associated with reproductive and physiological events occurring

in ewes during growth phase. According to Wankowska *et al.* (2008), in lambs, the mechanisms that control the release of GH are most active during the first phase of growth.

Hornick *et al.* (2000) state that, during the restricted feeding period, plasma GH concentration is usually high, inducing mobilization of adipose tissue, used to meet energy requirements for the growth phase.

Therefore, differences in plasma GH concentrations were expected, and Treatment A lambs, being the ones that would have the highest values during phase 1. However, despite the lower body development achieved by lambs of this treatment, the diet may not have been as strict and this, by itself, could not directly change the plasma GH concentration, confirming Trenkle (1974) who reported that less severe dietary restrictions do not affect the plasma GH concentration. Thus, plasma GH concentration in lambs on phase 1 depends on the intensity and duration of the restriction of the adopted food plan.

During Phase 2, plasma GH concentration remained similar between treatments. Thus, these GH concentrations reflected parallel physiological events on groups A and B.

## CONCLUSION

The application of a diet in order to obtain a lower growth rate in confined Bergamasca lambs during growth phase promotes satisfactory body development and age at onset of reproductive activity. Therefore, it can be indicated as an alternative feeding management for future replacement ewe lambs.

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