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DETERMINATION OF THE SUCKLING-RUMINANT PHASE ON LAMBS BY δ13C TECHNIQUE

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RESUMO

This study was conducted at Universidade de Marília, São Paulo State, Brazil, aiming to determine the phase of intake transition from exclusively liquid diet (milk) to diet based on milk, grass and/or ration in lambs by δ13C technique. Six Suffolk crossbreeding lambs were distributed in a totally random design (3x2), with two experimental groups and three replications. In the first group, lambs were penned and sucked milk from dams fed with C4 diet. besides, they had access to C3 ration in creep feeding, since their birth. In the second one, lambs were kept on a paddock with Tifton – 85 grass (C4 photosynthetic way), as well as their dams, and had access to C3 ration in creep feeding, since their birth. The C3 ration contained soybean meal, ground oat grains, lucerne hay and mineral salt. The penned lambs' ration consumption overcame the milk intake at 30-35 days after birth; however, grazing lambs ingested more milk and pasture than ration until 40 days after birth. The δ 13C technique was considered effective on studies of lambs feeding behavior.

KEYWORDS: Creep feeding, *Cynodon dactylon*, intake, stable isotopes, Suffolk.

ABSTRACT

DETERMINAÇÃO DA FASE LACTENTE-RUMINANTE DE CORDEIROS PELA TÉCNICA DO $\delta^{13}C$

O trabalho foi realizado na Universidade de Marília, SP, com o intuito de determinar, pela técnica do $\delta^{13}C$, a fase de transição do consumo da dieta exclusivamente líquida (leite) para dieta composta por leite, pasto e/ou ração em cordeiros. Distribuíram-se seis cordeiros mestiços Suffolk em delineamento inteiramente casualizado, em esquema fatorial (3x2), sendo dois grupos experimentais e três repetições. No primeiro grupo, os cordeiros foram mantidos confinados e mamavam leite de ovelhas alimentadas com dieta de característica C_4. Além disso, tiveram acesso à ração de caráter C_3 no *creep feeding*, desde o nascimento. No segundo grupo, os cordeiros foram mantidos em pastagem de Tifton-85 (ciclo fotossintético C_4) juntamente com as ovelhas, tendo acesso à ração de caráter C_3 no *creep feeding*, desde o nascimento. A ração de caráter C_3 do *creep feeding* continha farelo de soja, grãos de aveia triturados, feno de alfafa moído e sal mineral. O consumo de ração tornou-se superior à ingestão de leite no período de 30-35 dias após o nascimento dos cordeiros confinados, contudo, os cordeiros em pastejo ingeriram mais leite e pasto, em comparação à ração, até 40 dias após o nascimento. A técnica do $\delta^{13}C$ foi considerada eficaz nos estudos do comportamento alimentar de cordeiros.

KEYWORDS: Creep feeding, *Cynodon dactylon*, intake, stable isotopes, Suffolk.

INTRODUCTION

Creep feeding means supplying supplentary ration for suckling lambs in unaccessible areas for ewes (NRC, 1985). Such practice has minimized obstacles of sheep rearing in Brazil. It is opposed to the traditional finishing systems, decreases slaughter age of lambs and improves carcass quality. Considering the seasonality of forrage offer, especially in the periods of inferior pasture, creep feeding is essencial for lambs to reach heavier weight at weaning (MACEDO et al., 2000).

NERES et al. (2001), studying the levels of lucerne hay in creep feeding for crossbred Sulfolk lambs on Cynodon plectostachyus pasture, verified that animals which had access to creep feeding were weaned at 56 days, with live weight of 24.58 and 21.74 kg for males and females, respectively. On the other hand, animals without access to creep feeding were weaned with 18.30 and 16.81 kg for males and females, respectively.

There are no records in the literature, excepting bevarior observation data by JOHNSTON (1992), about the moment when lambs in creep feeding start ingesting solid food in significant amount so that it can surpass the milk contribution to the animal´s performance. Most of the authors agree that lambs start ingesting ration in creep feeding when they are around 10 to 14 days of age, and the quantity of ration ingested is inversely proportional to the quantity of milk ingested (NRC, 1985).

It is essencial to determine the most appropriate moment for the beginning of ration supply in creep feeding, in order to positively affect lambs´performance, minimize losses and rationalize the management of the animals feeding.

The use of fistulas could help this kind of studies, if there were no limitations regarding the costs, the necessity of surgical intervention, adaptation period of the animals, restrict number of animals per experiment, and changes in feeding habits of the animals. In this context, the carbon stable isotopes techinque (LUDLOW et al., 1976) has become an important instrument in the study of ruminants diet composition, especially because it is a quantitative method, the analysis has a non-invasive character and the execution is easy.

The δ13C technique is based on the isotope discrimination that occurs in plants during photosynthesis process. In C4 plants, in which photosynthesis occurs via carboxylic acid, the isotope abundance of 13C is around 12% to -14 ‰ (units of δ13C). In C3 Calvin-dependent plants, the values are around -26% to -28 ‰ (MINSON et al., 1975). As isotope markings are preserved during the passage of the food through the digestive tract of the animals, it is possible to estimate the proportion of C3 and C4 consumed by the animals by means of stool analysis. Therefore, the purpose of this research was to determine the transition phase from the intake of an exclusively liquid diet (milk) to a liquid-solid diet (milk, pasture and/or ration) in lambs, by the δ13C technique.

MATERIAL AND METHODS

The experiment was carried out at the ovine culture sector, of the Agricultural Science Faculty, Universidade de Marília (Marília University), in Marilia (22°13'10" Latitude South and 49°56'45" Longitude West), São Paulo, Brazil. The experimental period lasted 60 days for each lamb, counted from the birth.

Six crossbred Suffolk lambs, from simple or multiple birth, were used and allocated in a completely randomized experimental design (3x2), with three replications and two groups. In the first group, in a penned system, lambs suckled the milk from ewes fed diet composed of ingredients from the C4 photosynthetic cycle, and had access to creep feeding with C3-character ration. In the second group, lambs remained with the ewes on C4 photosynthetic cycle pasture, and had access to creep feeding with character C3 ration since birth.

The C4 pasture used was Tifton-85 (Cynodon dactylon), and the C3 ration of the creep feeding was composed of soybean meal, ground oat grain, ground lucerne hay and mineral salt. The ingredients proportion in the ration followed the requirement recommendations by NRC (1985). .

The C4-character diet of the ewes from the first group was composed of ground dry corn and urea mixed to Tifton – 85 hay and remained the same during all the experimental period. The ewes from the second group were kept on pasture on the same grass and received only the concentrate of ground dry corn and urea. The ewes were fed daily at 8:00 am and 2:00 pm, so that neither penned lambs nor the ones on pasture had access to the ewes´ ration.

Daily milkings were carried out to record the ewes´ milk production and to collect samples for isotopic analysis. Before such analysis, milk samples were dried in a hothouse with forced air circulation at 50°C for 48 hours, according to SILVA et al. (2002). Sampling was also carried out daily, directly from the lambs rectum, to avoid contamination. The samples were acconditioned in plastic bags duely identified, and were kept under refrigeration until it was ready for isotopic analysis.

Stool samples were dried in a hothouse with forced air circulation at 50°C for 48 hours. Samples corresponding to the first week of life of the lambs required more time in the hothouse, because, in this phase, lambs were suckling colostrum, which contains a high fat rate. After being withdrawn from the hothouse, the stool samples were pre-grounded in a porcelain crucible, and then each sample was submitted to the grinding process in a cryogenic mill (Spex 6700-230 Freezer/Mil Industries, Edisom, United States), at -196°C, for three minutes, in a high frequency, for obtaining homogeneous material with extremily thin granulometry and microscopic aspect.

For the measurement of the δ13C in the samples, around 0.2 mg of milk and 0.4 mg of feces were used. These samples were placed in a tin capsule and then they were introduced, by means of an automatic sampler, into an elemental analyzer (EA 1108–CHN–Fisions Instuments, Rodano, Italy), in which the sample was quantitatively burnt to CO_2, in the presence of oxygen (O_2) and copric oxide (CuO).

The gas obtained was separated in a gas chromatography column and analyzed in an isotope-ration mass spectrometer (Delta S–Finnigan Mat, Bremen, Germany). The value of the isotope ration is expressed in delta per thousand (δ‰), relative to the PDB international standard for 13C, according to the following general equation:

δ‰(sample, standard) = [(Rsample- Rstandard)/Rstandard] x 1000

In the expression cited above, R represents the ration between the heavier isotope and the lighter one, particularly 13C/12C for carbon. Each sample was analyzed twice for obtaining mean values. The measures were repeated when the analytical standard deviation was higher than 0.2‰. The obtained data were analyzed by the method of regression equations of the Origin® 6.0 Pro¬fessional software (MICROCAL SOFTWARE, 1999).

RESULTS AND DISCUSSION

The analysis of the δ‰13C of the feeding sources for both lambs and ewes is presented in Table 1.

Table 1. Mean values of δ‰13C and their respective standard deviations for the source of feed for ewes and lambs

Ingredients	δ‰^{13}C
Concentrate for ewes on pasture[1]	-12,66 ± 0,06
Penned ewes´diet[2]	-13,79 ± 0,10
Tifton – 85 pasture	-16,31 ± 0,08
Creep feeding ration	-26,86 ± 0,15
Milk of penned ewes	-14,25 ± 0,20
Milk of ewes on pasture	-15,20 ± 0,20

[1]Concentrate composed of ground corn, urea and mineral salt.

[2]Diet composed of ground corn, urea, mineral salt and Tifton-85 hay

The δ‰13C values of the pre-established diets for ewes and lambs fit the isotope abundance rates suggested by LUDLOW et al. (1976), -12% to -14 ‰, in which there is the C4- character diet for ewes, and -26% to -28 ‰, in which there is the ration for

lambs, composed of ingredients of exclusively C3 photosynthetic cycle.

Figure 1 illustrates the sigmoidal behavior of isotopic curves obtained from the δ‰13C values of the lambs´ feces of the frist group, which suckled milk (C4) and had access to the ration (C3) in creep feeding since birth.

According to what was described in a complementary research (SILVA et al., 2007), the model of Boltzmann´s sigmoidal regression was applied to the distribution of the δ13C values, which characterize the general average of the groups of lambs. It is the best model to adjust to the isotopic results, and the isotopic terminology was attributed to the general equation of the following model :

$$y = \frac{A_1 - A_2}{1 + e^{\frac{(x - x_o)}{dx}}}$$

, in which:
xo: sigmoid inflection point; dx: constant time; A1: initial value of the axle Y→ y (-∞); A2: final value of the axle Y→ y (+∞).

The Y value in x0 is half the distance between the two limiting values A1 and A2: y(x0) = (A1+A2)/2. The Y value changes drastically according to variations of X. The magnitude of such variations is approximatedly dx.

This way, it was observed that, by the δ‰13C mean value of the feces, distributed in five-consecutive days intervals, the prospect of the intake dynamics of the group of penned lambs confirms the importance of milk during the three first weeks of life and a significant intake of ration in creep feeding right after this period.

Probably, milk production of ewes after the third week of life of penned lambs was not enough to support the high demand, forcing the lambs to search for another feeding source. FOLMAN et al. (1966) stated that the supression of constant suckling can force the intake of concentrate food by the lamb.

SUSIN (1996) also considers the texture and palatability of the concentrate mixture as stimuli for the volunteer consumption of solid food by the lambs. Thus, the use of noble ingredients in the ration composition, such as soybean, lucerne and oat, associated to the decrease of milk production, could have influenced the increase of creep feeding ration intake from the 25th day on, so that, from the 30th-35th day of life the isotopic sign of penned lambs´feces was closer to the isotopic sign of the ration (-26.86 ‰), producing balance (Figure 2).

Individual isotopic curves of lambs which had accesss to the three sources of feeding (C4- character milk, C4 photosynthetic cycle pasture and ration constituted of C3 ingredients) and the δ‰13C mean values of the feces are presented in Figure 2.

The sigmoidal behavior of the isotopic curve was less accentuated for the groups of lambs in grazing due to the presence of the third feeding source, the pasture. Because of that, Figure 2 presents only one level (from birth to 25 days of age, with isotopic values close to the C4-character milk) and values tending to the ration isotopic sign (-26.86 ‰) from 25 to 60 days of age. Thus, there is no balance level close to the C3 ration sign, as it was observed for lambs in the second group (Figure 1).

Such fact shows that 60 days were not enough for the lambs that had access to the three feeding sources to prefer the intake of ration in creep feeding rather than ingesting milk and pasture. Therefore, learning to graze a few days after birth emphasizes the importance of offering good quality pasture, which, associated with milk, guarantees the nutritional support of lambs until the beginning of ration intake in creep feeding.

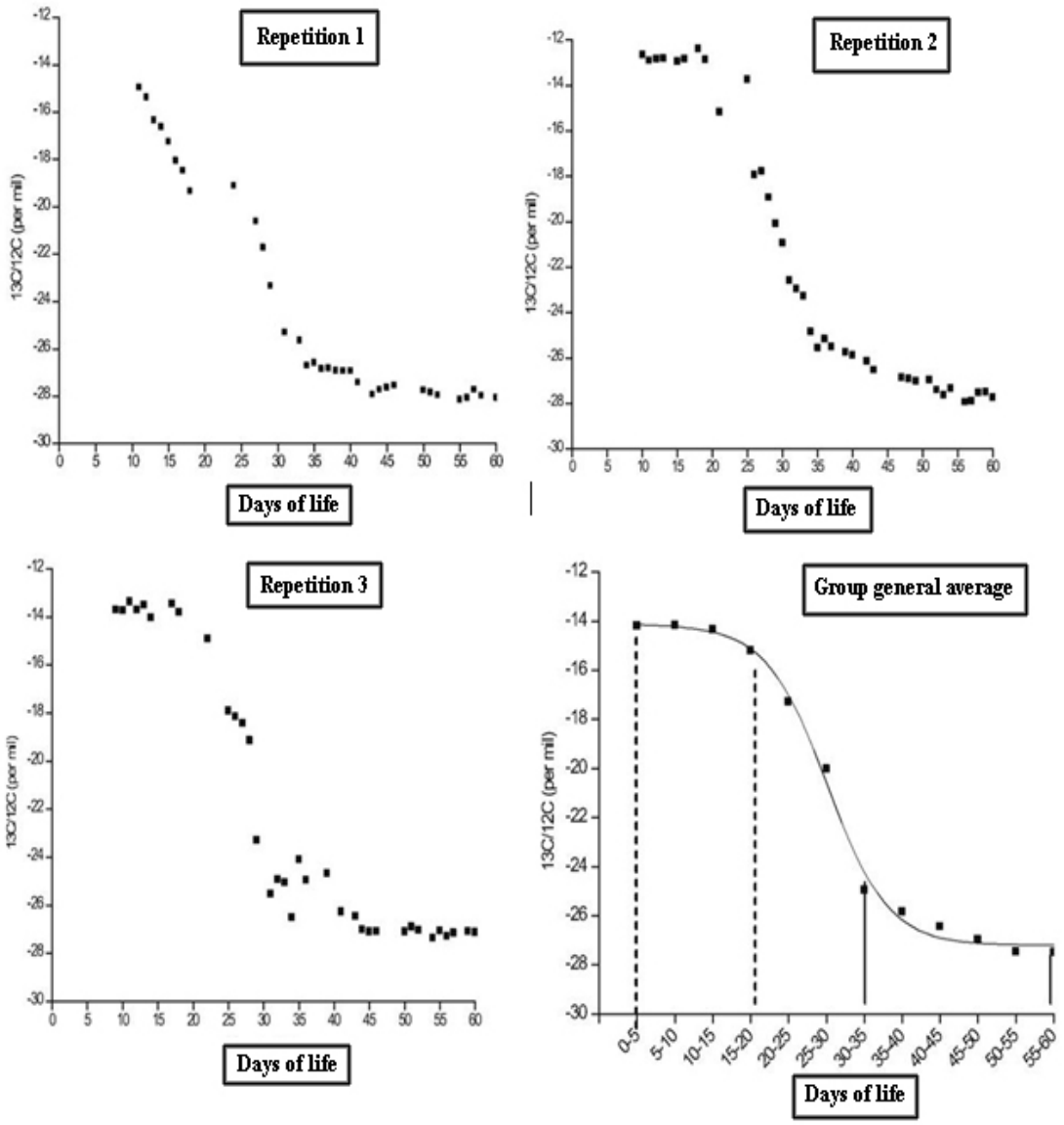

FIGURE 1. Values of δ‰13C of the feces of penned lambs, fed with milk from ewes fed with C4-character diet and with access to C3-character ration in creep feeding, since birth. (: :) Traced lines limit the area correspondent to the period of the first three weeks of the lambs´lives and the almost milk-exclusively intake. (l l) Continuous lines record the start of the ingestion of solid-exclusively diet (30-35 days of life) and the subsequent replacement of milk by ration in creep feeding.

Model: Boltzmann (Ch^2 = 0.18102 / R^2 = 0.99612)

A1 -14.14067 ± 0.26787
A2 -27.22083 ± 0.22979
x0 6.02077 ± 0.09308
dx 0.80752 ± 0.08317

$$Y = \frac{-14.14067 - (-27.22083)}{1+e^{(X-6.02077)/0.80752}}$$

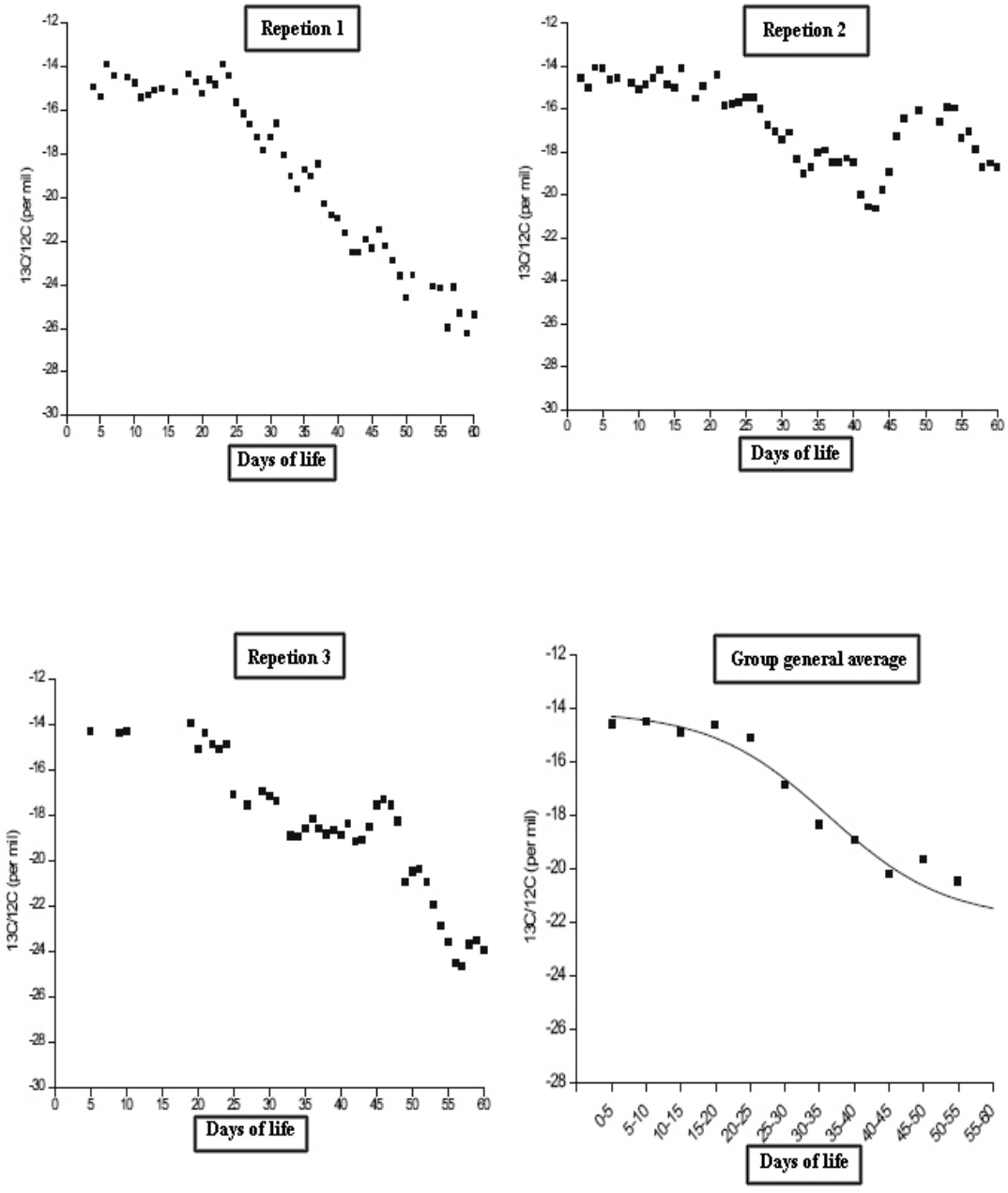

FIGURE 2. Values of δ‰13C of the feces of lambs on Tifton-85 (C4) pasture, fed with milk from ewes fed with C4- character diet and with access to C3-character ration in creep feeding, since birth. (::) Traced lines limit the area correspondent to the period of the first three weeks of the lambs´lives and the almost milk-exclusively intake. Subsequent area corresponds to the simultaneous ingestion of the three sources: milk, pasture and ration in creep feeding.

Modelo: Boltzmann (Ch^2 = 0.48147 / R^2 = 0.95581)

A1 -14.13122 ± 0.70925
A2 -21.93565 ± 1.05158
x0 8.24502 ± 0.61598
dx 1.69351 ± 0.66237

$$Y = \frac{-14.13122 - (-21.93565)}{1+e^{(X-8.24502)/1.69351}}$$

CONCLUSIONS

The ingestion of milk associated with grazing high quality forrages guarantees the development of lambs raised on pasture and conditions them to a later ration intake in creep feeding (from 40-45 days of age). On the other hand, penned lambs without access to pasture search solid food precociously (from 30-35 days of age), because these conditions require more milk production from the ewes.

This way, isotopic analysis of feces is a viable tool for studies regarding the composition of ovine diet, because it reliably reflects which kind of food is ingested without interfering in the animals´selectivity.

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