Effect of Feeding Frequency on Intake, Digestibility, Ingestive Behavior, Performance, Carcass Characteristics, and Meat Quality of Male Feedlot Lambs

Rodrigo B. Saldanha, Ana C. P. dos Santos, Henry D. R. Alba, Carlindo S. Rodrigues, Douglas dos S. Pina, Luis G. A. Cirne, Stefanie A. Santos, Aureliano J. V. Pires, Robério R. Silva, Manuela S. L. Tosto, Silvia C. Bento, Amanda B. Grimaldi, Carly A. Becker and Gleidson G. P. de Carvalho

Abstract: The study was conducted to evaluate the effects of feeding frequencies (one, two, three, and four times per day) on the performance of male feedlot lambs. Forty Santa Inês male lambs were used with an average age of 120 days approximately and initial body weight of 24.90 ± 1.8 kg (mean ± S.D.). The lambs were distributed to treatment groups in a completely randomized experimental design, with 10 animals per treatment, placed in individual pens. A standard diet consisted of Tifton-85 hay and concentrate with a ratio of 60:40, respectively. The different feeding frequencies did not influence (p > 0.05) the intake of nutritional components, blood parameters, weight gain, ingestive behavior, carcass characteristics, or meat quality of male feedlot lambs. The feeding frequency of 3 and 4 times per day promoted the highest digestibility of neutral detergent fiber (NDF; p = 0.008) and total digestible nutrients (TDN; p = 0.002). The feeding frequency of 3 times per day promoted the highest digestibility of crude protein (CP; p = 0.005). The time devoted to rumination (min/kg DM) was increased (p = 0.029) when the diet was supplied once a day compared to the other feeding frequencies. Based on these results, the recommended feeding frequency for male feedlot lambs is once a day. When feeding at this frequency, the ingestive behavior, productivity, carcass characteristics, and meat quality of male feedlot lambs will not be compromised.

Keywords: feeding frequency; intensive system; ruminant nutrition; sheep

1. Introduction

One of the challenges of sheep farming is meeting the demanding consumer market for quality meat. Researchers have reported that intensive systems are an effective method for raising feedlot lambs. This system shortens the production cycle allowing for increased carcasses processed in the same amount of time and better quality meat [1–3].

Animal scientists have discovered new feed ingredients for use in the diets of lambs [4–6], which has contributed to greater weight gains and improved meat quality. However, the scope of productive efficiency in feedlot animals also depends on other factors, including the strategy of how the diet is fed [2].
The purpose of intensive lamb systems is to satisfy the feeding requirements of the animals; however, a lack of standardization of strategies in the frequency of feeding the diet exists. Studies on feeding frequency in large ruminants, specifically dairy cattle [7–10], are available. However, in small ruminants, the scientific evidence to elucidate the appropriate feeding frequency in lambs is limited [11–14].

In studies with dairy cows, higher feeding frequencies promoted a higher intake of nutritional components and milk production [15]. The physiology and behavior of small ruminants is different from that of large ruminants [16,17], which indicates the importance and need to determine feeding frequency strategies in sheep production that could improve performance measures.

Santa Inês is the result of the intercurrent crossing in the northeast of Brazil between the genetic groups Bergamácia, Morada Nova, Rabo Largo, Somális, and other sheep without a defined racial pattern; it stands out for its productivity and rusticity, being biologically considered a new genetic material [18]. Male lambs of the Santa Inês breed show efficient growth parameters from 150 (extensive systems) to 250 (intensive systems—feedlot) grams/day approximately [19,20]. In an intensive system, male Texel x Santa Inês crossbreed lambs showed a 65% difference in slaughter age (159 days—pasture vs. 263 days—feedlot) and 116% in average daily gain (0.133 g—pasture vs. 0.288 g—feedlot) [21].

Thus, the authors hypothesized that by increasing the frequency of feeding diets throughout the day, this could positively stimulate the productive and behavioral characteristics of feedlot lambs to diets that consist of hay and concentrate, in addition to promoting better quality meat.

Thereby, the objective of this study was to evaluate the intake, digestibility, ingestive behavior, performance, carcass characteristics, and meat quality of feedlot lambs subjected to four different feeding frequencies.

2. Materials and Methods
2.1. Local and Ethics Committee

The experiment was carried out at the Experimental Farm of the Federal University of Bahia, located in the municipality of São Gonçalo dos Campos, Bahia, Brazil, at 12°23′57.51″ south latitude and 38°52′44.66″ west longitude. The farm is located in the Recôncavo Baiano region and has annual averages of 26 °C in temperature, 85% relative humidity, and annual rainfall of approximately 1200 mm.

All the procedures and protocols used in this experiment were approved by the Animal Use Ethics Committee of the Federal University of Bahia (approval number: 18/2015).

2.2. Animals, Treatments, and Experimental Managements

Forty Santa Inês, uncastrated male lambs were used for this study. The lambs were on average 120 days old approximately with an average body weight (BW) of 24.90 ± 1.8 kg (mean ± S.D.). The lambs were vaccinated against rabies and clostridial infections (Gepec S.A., Belo Horizonte—M.G., Brazil), de-wormed (albendazole, 2.5 mg/kg BW—Aldazol®, Vallée S.A., São Paulo, Brazil), and housed in individual pens (1.2 m²), covered, with a slatted floor, and suspended (1 m raised off of the ground). Each pen had access to an individual feeder and drinker.

The study consisted of four treatment groups with 4 different feeding frequencies, one time per day, two times per day, three times per day, and 4 times per day. The experiment consisted of 85 days, with 15 days of adaptation to the environment, management, and diets, and 70 days of data collection. The adaptation period was used to determine the feed intake and, thus, be able to start the experimental period with the real knowledge of feed intake.

The feeding frequency was as follows: one time per day = 100% of feed delivered at 8:00 h; two times per day = 50% of feed at 8:00 h and 50% at 14:00 h; three times per day = 34% of feed at 8:00 h, 33% at 1100 h, and 33% at 14:00 h; four times per day = 25% at 8:00 h, 25% at 11:00 h, 25% at 14:00 h, and 25% at 17:00 h. In the current experiment,
lambs had free access to water. Furthermore, in all strategies, diets were provided to allow for 10 to 20% of refusals (on an as-fed basis) to ensure voluntary intake. Each treatment was repeated 10 times (10 lambs on each treatment). The lambs were randomly assigned treatments in a completely randomized experimental design.

The lambs were being fed the same experimental diet (Table 1) that consisted of a concentrate mix (ground corn, soybean meal, urea, and a sheep mineral mix) concentrate and a previously ground Tifton-85 (Cynodon sp.) hay. The diet was formulated to meet the requirements of growing lambs with a weight gain of 200 g/day following guidelines from the National Research Council [22].

Table 1. Proportion of ingredients and chemical composition of experimental diet.

| Ingredient          | g/kg Dry Matter |
|---------------------|-----------------|
| Soybean meal        | 245.0           |
| Ground corn         | 234.0           |
| Mineral mixture     | 15.0            |
| Urea                | 6.0             |
| Tifton-85 hay       | 500.0           |

| Composition Chemical | g/kg Dry Matter |
|----------------------|-----------------|
| Dry matter (g/kg as-fed) | 880.1          |
| Ash                  | 53.9            |
| Crude protein        | 152.0           |
| Ether extract        | 15.0            |
| Neutral detergent fiber | 419.3          |
| Non-fibrous carbohydrates | 251.7          |
| Total digestible nutrients | 695.9          |

1 Guarantee levels (per kg of active elements): calcium 120.0 g; phosphorus 87.0 g; sodium 147.0 g; sulfur 18.0 g; copper 590.0 mg; cobalt 40.0 mg; chromium 20.0 mg; iron 1800.0 mg; iodine 80.0 mg; manganese 1300.0 mg; selenium, 15.0 mg; zinc 3800.0 mg; molybdenum 300.0 mg; fluorine 870.0 mg.

2.3. Data Collection and Chemical Analyses

Weekly samples of ingredients, diets, and refusals were collected and stored in plastic containers in a freezer at −20 °C. At the end of the experiment, the samples were thawed at room temperature and pre-dried in a forced ventilation oven at 55 °C for 72 h and ground in a Willey mill (Tecnal, Piracicaba, São Paulo, Brazil) with a screen fitted with 1 mm sieves. Portions of the original samples were prepared and used in the laboratory analysis.

The analysis of dry matter (DM; method 920.15), ash (method 942.05), crude protein (CP = nitrogen × 6.25; method 948.13), and ether extract (EE; method 920.39) were performed according to the AOAC [23]. The organic matter (OM) was determined by the difference between the content of DM and ash. The neutral detergent fiber (NDF) was determined according to the specifications described by Mertens [24] and Licitra et al. [25]. Non-fibrous carbohydrates (NFC) were calculated according to Hall [26]: NFC = 100 − [(%CPdiet − %CPurea + %Ureadiet) + %EE + %NDF + %Ash]. The TDN was calculated according to da Cruz et al. [27].

2.4. Intake and Apparent Digestibility of Nutrients

The weights of feed supplied and the refusals weights were recorded daily to determine the intake of nutritional components. The digestibility tests were carried out between days 30 and 37 and between days 60 and 67 of the experimental period, by collecting all of the feces for the respective days. After 24 h of each collection day, the feces samples were weighed. Next, aliquots of 10% of the total excreted feces were pre-dried in a forced ventilation oven (55 °C for 72 h) and ground in a Willey-type mill with a 1 mm sieve. Composite samples for each animal were prepared and stored for further analysis.

The apparent digestibility coefficients (DC) of DM, ash, CP, EE, NDF, and NFC were calculated using the following equation: DC = [(Amount ingested − Amount excreted)/(Amount ingested)] × 100.
2.5. Ingestive Behavior

All lambs were subjected to visual observation to evaluate ingestive behavior on days 21 and 59 of the experimental period. Observations were made every five minutes during a 24 h period to determine the time spent on feeding, rumination, and idleness activities [28]. On the same day, the number of chews (NC) was recorded, and the rumination time of each ruminal bolus was obtained (three evaluations per animal) in three different periods of the day (1000 h to 1200 h; 1400 h to 1600 h; 1900 h to 2100 h) using a digital stopwatch. All other parameters of ingestive behavior were calculated according to the procedures described in Polli et al. [29] and Bürger et al. [30].

2.6. Blood Metabolites and Hepatic Enzyme Activity

On day 67 of the experimental period, 10 mL of blood was drawn (four hours after the first feeding) into tubes without anticoagulant (BD Vacutainer® SST II Advance, São Paulo, Brazil) by jugular vein puncture. The samples were centrifuged at 1500 rpm × g for 15 min to obtain the serum. The serum was then transferred to eppendorf microtubes (Sigma-Aldrich Brasil Ltd.a., Duque de Caxias, SP, Brazil) and stored in a −20 °C freezer for further analysis.

The concentrations of urea and total proteins (TP), as well as the activities of the enzymes to assess liver metabolism, aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT), were measured in a kinetic system using commercial kits (doles®, São Paulo, Brazil). All blood metabolite readings were performed using a semi-automatic spectrophotometer (SBA 200®, CELM, São Caetano do Sul, Brazil) according to the respective wavelengths. The analyses were carried out at the Animal Nutrition Laboratory (LANA) of the Federal University of Bahia (UFBA).

2.7. Growth Performance

The animals were weighed individually in the morning, after solids fasting for approximately 16 h, at the beginning (day 1) and the end (day 71) of the experimental period. Total weight gain (TWG) was calculated by the difference between final body weight (FBW) and initial body weight (IBW). The average daily gain (ADG) was obtained from the TWG divided by the number of days the animals were in confinement (70 days). The feed conversion (FC) was calculated by dividing the DM intake (g/day) by the ADG (g/day).

2.8. Slaughter and Carcass Characteristics

At the end of the experiment, the animals were sent to a commercial slaughterhouse, where they were reweighed to obtain the slaughter body weight (SBW). At the time of slaughter, the animals were stunned by electronarcosis, followed by bleeding, skinning, evisceration, and removal of the head and limbs. After slaughter, the carcasses were weighed to obtain the weight (HCW) and yield (HCY = HCW/SBW × 100) of the hot carcass.

After the carcasses remained 24 h at 4 °C, the carcasses were subjectively evaluated by a trained evaluator in terms of conformation, finishing, fattening, and marbling using a scale with scores from 1 to 5 according to Cézar and Sousa [31]. The following morphometric measurements were determined: external and internal length of the carcass; width, depth, and perimeter of the chest; rump width and circumference; leg length and circumference. Length and circumference measurements were taken with a tape measure, and width and depth measurements were taken with a handheld hypometer (Walmur, Salvador, Bahia, Brazil).

Immediately after the carcasses were evaluated, they were sectioned in half. The left half was subdivided into five anatomical regions considered commercial cuts (neck, shoulder, loin, rib, and leg), which were weighed individually to determine the yields. The loins of each animal were packed, identified, and frozen (−20 °C) for the subsequent analysis of color, cooking losses, shear force, and proximate composition.
2.9. Physicochemical Characteristics and Centesimal Composition of the Meat

The loins were thawed in plastic bags in a Bio-Oxygen Demand incubator (Tecnal, Piracicaba, São Paulo, Brazil) at 10 °C for 12 h. The loins were then dissected with a scalpel and a knife. On the left spine, the color was determined with a Minolta CR-400 colorimeter (Konica Minolta, Osaka, Japan), using the CIELAB L* system (brightness), a* (redness), and b* (yellowness). The colorimeter was calibrated with an illuminated white ceramic plate for standard observation and operated using an open cone. The color was determined five minutes after the cross-section of the exposed sample. After this period and according to Miltenburg et al. [32], the coordinates L*, a*, and b* were measured at three different points on the internal surface of the muscle.

Cooking losses (CL) were determined on loin samples that were approximately 1.5 cm thick, 3.0 cm length, and 2.5 cm wide. The meat samples were weighed, placed on an aluminum-lined tray, and cooked in an oven preheated at 170 °C until the center of the meat reached a temperature of 70 °C, and measured by a copper-constantan thermocouple equipped with a digital reader (Delta OHM, model HD9218, Caselle di Selvazzano PD, Italy). The samples were cooled to room temperature and reweighed. Cooking losses were calculated as the difference in weight before and after heat treatment [33].

Shear force (FC) was determined using the same cooked meat samples that were used to measure cooking losses. Each sample was cut into three 25 mm × 25 mm × 20 mm cubes and sectioned in the transverse direction of the muscle fibers to determine the tenderness of the meat using a “texturometer” texture analyzer (Stable Micro System, Surrey, United Kingdom), adopting the method described by Wheeler et al. [34].

The proximate composition (dry matter, moisture, protein, and fat) and collagen of the meat were measured by near-infrared spectroscopy (method: 2007-04) [35] in 180 g of *Longissimus lumborum* muscle without covering fat, using the FoodScanTM Diagnostic (FOSS, Hillerod, Denmark) with an artificial neural network calibration model and associated database. The ash content was determined after the ignition of a meat sample in a muffle at 550 °C [36].

2.10. Statistical Analyses

The results were evaluated by analysis of variance and Tukey’s test. For the current study data (intake, digestibility, ingestive behavior, meat yield, carcass characteristics, and meat quality), the statistical model included feeding frequency as fixed effect, and lamb was considered as random effect. For this, PROC MIXED of SAS 9.4 (SAS Institute Inc., Cary, NC, United States) was used, according to the statistical model below:

\[
Y_{ij} = \mu + F_i + \epsilon_{ij},
\]

where \(Y_{ij}\) = dependent variable; \(\mu\) = mean; \(F_i\) = fixed effect of the feeding frequency (i = 1, 2, 3, and 4 times a day); \(\epsilon_{ij}\) = assumed random error NID ~ (0, \(\sigma^2\)). For all comparisons, a 5% probability of type I error was considered.

No animals died or suffered accidents during the experiment. Furthermore, no outliers were observed in the data analysis.

3. Results

The frequency of feeding the diet did not affect nutrient intake (\(p > 0.05\)). The digestibility of DM, OM, EE, and NFC did not differ (\(p > 0.05\)) between the feeding frequencies of the diet. However, the 3- and 4-times-a-day feeding frequencies promoted an increased digestibility of NDF (\(p = 0.008\)) and TDN (\(p = 0.002\)) compared to the 1- and 2-times-per-day feeding frequencies. The lambs in the 3-times-per-day feeding frequency treatment had increased CP digestibility (\(p = 0.005\); Table 2) compared to the other feeding frequencies. No differences were observed in FBW (\(p = 0.881\)), TWG (\(p = 0.875\)), ADG (\(p = 0.869\)), and FC (\(p = 0.894\)) with the different feeding frequencies.
Table 2. Intake and digestibility of nutritional components and performance of male feedlot lambs subjected to different feeding frequencies.

| Items                              | Feeding Frequency (Number/Day) | SEM ¹ | p-Value ² |
|------------------------------------|--------------------------------|-------|-----------|
|                                    | 1    | 2    | 3    | 4    |       |        |
| Intake (kg/day)                    |      |      |      |      |       |        |
| Dry matter                         | 1.006| 1.058| 1.130| 1.238| 0.07  | 0.137  |
| Organic matter                     | 0.913| 0.980| 1.060| 1.089| 0.06  | 0.180  |
| Crude protein                      | 0.259| 0.271| 0.291| 0.310| 0.02  | 0.172  |
| Ether extract                      | 0.017| 0.018| 0.020| 0.021| 0.00  | 0.139  |
| Neutral detergent fiber            | 0.394| 0.422| 0.448| 0.492| 0.03  | 0.083  |
| Non-fibrous carbohydrates          | 0.263| 0.273| 0.287| 0.316| 0.02  | 0.203  |
| Apparent total tract digestibility (%) |      |      |      |      |       |        |
| Dry matter                         | 73.28| 76.58| 82.24| 82.2 | 2.91  | 0.161  |
| Organic matter                     | 74.28| 78.68| 81.98| 81.0 | 2.51  | 0.051  |
| Crude protein                       | 83.42| 86.99| 91.88| 89.31| 1.96  | 0.005  |
| Ether extract                       | 73.54| 83.59| 76.09| 77.77| 2.79  | 0.026  |
| Neutral detergent fiber            | 60.99| 69.63| 73.47| 72.18| 3.22  | 0.008  |
| Non-fibrous carbohydrates          | 87.49| 91.25| 87.40| 90.12| 1.79  | 0.232  |
| Total digestible nutrients         | 69.59| 74.74| 80.29| 80.31| 3.29  | 0.002  |
| Performance (kg)                   |      |      |      |      |       |        |
| Initial body weight                | 24.95| 25.77| 24.91| 24.21| 0.90  | -      |
| Final body weight                  | 36.91| 38.39| 37.89| 37.41| 1.56  | 0.881  |
| Total weight gain                  | 11.96| 13.16| 12.98| 13.20| 0.97  | 0.875  |
| Average daily gain (g/day)         | 0.171| 0.188| 0.185| 0.190| 0.01  | 0.869  |

¹ SEM—standard error of the mean; ² p-value—means followed by different lower case letters differ statistically by Tukey test at 5% probability; ³ feed conversion in kg of dry matter intake/kg gain.

Feeding, inactivity, chewing times, feeding efficiency, and rumination efficiency were not influenced by feeding frequency (p > 0.05; Table 3). However, the time spent on rumination (Min/kg DM) in the lambs fed once per day was greater (p = 0.029) than the time spent by the animals that received the diets more than once throughout the day (Table 3).

Table 3. Feeding behavior of male feedlot lambs subjected to different feeding frequencies.

| Items ¹                    | Feeding Frequency (Number/Day) | SEM ² | p-Value ³ |
|----------------------------|--------------------------------|-------|-----------|
|                            | 1    | 2    | 3    | 4    |       |        |
| Feeding                    |      |      |      |      |       |        |
| Minutes/day                | 287.9| 289.6| 290.5| 305.7| 14.44 | 0.750  |
| Minutes/kg DM              | 292.4| 288.4| 262.1| 264.9| 22.64 | 0.633  |
| Minutes/kg NDF             | 744.7| 717.7| 657.1| 665.0| 55.22 | 0.546  |
| Ruminating                 |      |      |      |      |       |        |
| Minutes/day                | 505.7| 452.9| 456.5| 486.0| 25.42 | 0.325  |
| Minutes/kg DM              | 502.7| 439.4| 407.6| 401.5| 28.85 | 0.029  |
| Minutes/kg NDF             | 1210.2| 1099.5| 1023.8| 1010.2| 72.04 | 0.147  |
| Idleness                   |      |      |      |      |       |        |
| Minutes/day                | 638.2| 697.9| 693.6| 634.0| 33.04 | 0.245  |
| Chews                      | 63.5 | 57.9 | 66.3 | 64.9 | 2.83  | 0.164  |
| Seconds/bolus              | 47.8 | 44.3 | 46.1 | 43.5 | 2.99  | 0.768  |
| Number/day                 | 29.473| 30.973| 29.236| 27.521| 30.95 | 0.716  |
| Hours/day                  | 17.8 | 18.6 | 19.9 | 21.6 | 0.55  | 0.127  |
| Minutes/kg DM              | 768.7| 690.2| 669.7| 666.4| 47.04 | 0.206  |
| Minutes/kg NDF             | 1955 | 1817 | 1681 | 1676 | 115.74| 0.183  |
| Feeding efficiency         |      |      |      |      |       |        |
| g DM/hour                  | 210.8| 207.5| 236.7| 226.5| 16.85 | 0.544  |
| g NDF/hour                 | 82.3 | 83.3 | 94.0 | 89.9 | 6.37  | 0.488  |
| Ruminating efficiency      |      |      |      |      |       |        |
| g DM/hour                  | 123.7| 140.4| 150.4| 144.4| 8.89  | 0.139  |
| g NDF/hour                 | 48.6 | 56.3 | 59.8 | 57.3 | 3.47  | 0.117  |
| g DM/bolus                 | 1.59 | 1.73 | 1.92 | 1.74 | 0.18  | 0.535  |
| g NDF/bolus                | 0.629| 0.695| 0.768| 0.691| 0.07  | 0.541  |
| Bolus (number/day)         | 684.3| 650.8| 625.4| 688.1| 59.28 | 0.853  |

¹ DM—dry matter; NDF—neutral detergent fiber; ² SEM—standard error of the mean; ³ p-value—means followed by different letters differ statistically by Tukey test at 5% probability.
Similarly, carcass weight and yield, commercial cut yield, subjective measures (conformation, finishing, fattening, and marbling), morphometric measures, and loin eye area were not affected \((p > 0.05)\) by the feeding frequency. However, the subcutaneous fat thickness was decreased \((p = 0.009)\) with the feeding frequency of one time per day (Table 4).

**Table 4.** Quantitative, subjective, and morphometric measurements of the carcass of male feedlot lambs subjected to different frequencies.

| Items                        | Feeding Frequency (Number/Day) | SEM 1 | \(p\)-Value 2 |
|------------------------------|-------------------------------|-------|---------------|
| Weight (kg)                  | 1                            | 2     | 3             | 4     |
| Slaughter                    | 36.02                         | 37.04 | 38.26         | 38.46 | 1.86 | 0.799 |
| Hot carcass                  | 16.80                         | 17.13 | 17.70         | 17.57 | 0.95 | 0.898 |
| Cold carcass                 | 16.71                         | 17.07 | 17.62         | 17.17 | 0.97 | 0.922 |
| Yield (%)                    | 46.60                         | 46.23 | 46.30         | 46.48 | 0.88 | 0.984 |
| Hot carcass                  | 45.69                         | 46.05 | 46.10         | 46.50 | 0.69 | 0.879 |
| Cold carcass                 |                              |       |               |       |      |       |
| Subcutaneous fat thickness   |                              | 1.22b | 1.66a         | 1.41ab| 1.58a| 0.19 | 0.009 |
| Morphometric measurements (cm) |                              |       |               |       |      |       |
| Internal length              | 68.45                         | 68.70 | 69.00         | 67.20 | 1.20 | 0.744 |
| External length              | 60.00                         | 59.60 | 59.80         | 58.62 | 0.96 | 0.714 |
| Leg length                   | 35.11                         | 34.50 | 34.30         | 34.50 | 0.67 | 0.866 |
| Leg circumference            | 34.50                         | 34.40 | 34.10         | 34.10 | 0.89 | 0.983 |
| Rump width                   | 26.00                         | 25.30 | 25.50         | 25.00 | 0.61 | 0.744 |
| Chest width                  | 20.00                         | 20.30 | 19.70         | 20.20 | 0.80 | 0.925 |
| Chest depth                  | 23.12                         | 22.90 | 23.80         | 22.44 | 2.73 | 0.117 |
| Rump circumference           | 63.45                         | 65.50 | 67.60         | 68.11 | 3.71 | 0.206 |
| Chest perimeter              | 72.25                         | 73.55 | 74.00         | 74.90 | 1.54 | 0.578 |

1 SEM—standard error of the mean; 2 \(p\)-value—means followed by different letters differ statistically by Tukey test at 5% probability; 3 SFT—subcutaneous fat thickness.

The variables related to the physicochemical characteristics and proximate composition of the *Longissimus lumborum* muscle were not affected \((p > 0.05)\) by the feeding frequencies (Table 5).

**Table 5.** Physicochemical characteristics and proximate composition of the *Longissimus lumborum* muscle of lambs subjected to different feeding frequencies.

| Items                        | Feeding Frequency (Number/Day) | SEM 1 | \(p\)-Value 2 |
|------------------------------|-------------------------------|-------|---------------|
| Color                        |                              |       |               |
| Brightness (L*)              | 46.69                         | 48.70 | 50.12         | 50.43 | 1.27 | 0.103 |
| Redness (a*)                 | 25.91                         | 26.29 | 27.70         | 26.33 | 0.68 | 0.320 |
| Yellowness (b*)              | 9.46                          | 10.16 | 11.15         | 11.57 | 0.62 | 0.074 |
| Cooking losses (%)           | 17.74                         | 16.41 | 18.26         | 16.94 | 2.55 | 0.941 |
| Shear force (kgf/cm²)        | 1.17                          | 1.06  | 1.16          | 1.19  | 0.12 | 0.882 |
| Proximate composition (%)    |                              |       |               |
| Moisture                     | 73.07                         | 72.94 | 70.77         | 72.52 | 0.62 | 0.088 |
| Protein                      | 22.13                         | 22.13 | 23.31         | 22.00 | 0.31 | 0.948 |
| Dry matter                   | 1.20                          | 0.99  | 1.10          | 1.18  | 0.16 | 0.852 |
| Fat                          | 3.79                          | 4.10  | 5.41          | 4.35  | 0.48 | 0.206 |
| Collagen                     | 1.44                          | 1.90  | 1.45          | 1.51  | 0.14 | 0.158 |

1 SEM—standard error of the mean.
Blood metabolites and liver enzyme activity were not altered by feeding frequency \((p > 0.05; \text{Table 6})\).

### Table 6. Blood metabolites and liver enzyme activity of male feedlot lambs subjected to different feeding frequencies.

| Items                                    | Feeding Frequency (Number/Day) | SEM \(^1\) | \(p\)-Value |
|------------------------------------------|-------------------------------|-----------|-------------|
| Blood metabolites and liver enzyme activity (UI/L) |                                |           |             |
| Urea (mg/dL)                             | 60.00                         | 3.08      | 0.629       |
|                                          | 58.60                         |           |             |
|                                          | 56.11                         |           |             |
|                                          | 55.11                         |           |             |
| Total proteins (g/dL)                    | 5.48                          | 0.35      | 0.081       |
|                                          | 5.18                          |           |             |
|                                          | 5.26                          |           |             |
|                                          | 5.67                          |           |             |
| Gamma-glutamyl transferase               | 52.35                         | 2.67      | 0.477       |
|                                          | 57.66                         |           |             |
|                                          | 58.33                         |           |             |
|                                          | 56.21                         |           |             |
| Aspartate aminotransferase               | 89.11                         | 6.01      | 0.052       |
|                                          | 91.20                         |           |             |
|                                          | 110.10                        |           |             |
|                                          | 93.89                         |           |             |

\(^1\) SEM—standard error of the mean.

### 4. Discussion

Several factors can affect feed intake, including the level or source of dietary fat \([1,3]\), the use or not of by-products \([37–40]\), the proportion of concentrate \([41]\), and the diet supply strategy \([9]\).

Feeding frequency increases the time spent feeding by lactating cows \([42]\). Thus, higher feed intake is expected in animals in which the diet was supplied more frequently during the day. In total mixed rations, higher diet intake is observed when using hay in sheep diets \([41]\), probably due to its physical and nutritional characteristics. Although hay was used in the present study, the diet was adjusted daily to ensure voluntary intake. Therefore, the amount of feed was not a limitation for intake regardless of the feeding frequency. In agreement to the results obtained, Ribeiro et al. \([43]\) did not observe differences in the time spent on feeding behavior activities (feeding, rumination, and idling) of male Santa Ines crossbred lambs subjected to three different feeding frequencies.

In cattle, the feeding frequency did not promote differences in nutrient digestibility \([9,44]\); however, in the current experiment, the researchers observed that the feeding frequency of 3 and 4 times per day resulted in increased digestibility coefficients for NDF and TDN. This effect may have occurred due to decreasing the meal size. Due to the smaller meal size, the number of ruminal microorganisms may have been increased; therefore, a larger distribution area for feed degradation was present \([45]\). All of this leading to an increased digestibility of NDF and, thereby, positively influencing the TDN values.

At the feeding frequencies of 1 and 2 times per day, the lambs that had decreased amounts of NDF may have degraded. This may have occurred by having a smaller number of microorganisms for a greater accumulation of fiber \([46,47]\), which leads to the lower digestibility of this nutrient.

The decreased amount of NDF per meal supplied with the feeding frequency of 4 times per day, reduced rumination time \(\text{Table 3}\), decreased salivation, ruminal buffering capacity, and consequently rumen pH \([48–50]\). The reduction in pH may have affected the fibrolytic bacteria in the rumen, which are the main bacteria responsible for protein degradability \([51,52]\), thus affecting the digestibility of this nutrient. Notably, this effect was not remarkably significant to affect NDF intake.

The greater action of ruminal bacteria on the feed at greater feeding frequencies resulted in a shorter rumination time, which can be corroborated by the results observed in the feeding behavior of the animals. Thus, it is observed that the smaller number of feeding times per day showed a greater time for rumination. The changes observed in intake, digestibility, and feeding behavior were not significant to influence the other factors of feeding behavior.

Feeding behavior can be affected by the level of dietary fiber, effective fiber, among others \([47,53,54]\). Therefore, as a standard diet was used and the intake rate was similar, the feeding behavior of the animals as a function of the feeding frequencies was similar.

The intensive system of continuous pens could have contributed to the results. As the diets were delivered to the pens, all of the lambs were alert and encouraged to approach their respective feeders, even if the diet was not provided for specific treatment pens.
at that time [55]. The results found can probably be extrapolated to systems of animals housed in groups, considering that this system has more effect on the behavior than on the performance of feedlot lambs [56].

Feed intake is one of the main factors affecting the performance of growing animals [57]. Since nutrient intake was not affected by feeding frequency in the present study, it was expected that the animals performed similarly.

As no differences were observed in the feeding behavior of the animals, probably, the similarity between the quantitative, subjective, and morphometric measures of the carcass and the physical–chemical characteristics and proximate composition of the Longissimus lumborum muscle of the lambs is due to similar characteristics between animals (age, gender, body weight, and phenotype).

In animal growth, subcutaneous fat is the first to accumulate in the body [58,59]. Acetate for the synthesis of fats in ruminants is a product of the degradability of carbohydrates in the diet [60,61]. In the present study, lambs fed one time per day had the lowest NDF digestibility values. Thus, the lower digestibility of NDF and the consequent lower production of acetate may have reduced the available substrate for fat synthesis. Due to this, the lambs being fed one time per day had less subcutaneous fat compared to the lambs that were fed more than once a day.

Results similar to those of the present study were observed in studies with sheep [62] and cattle [7,9,63]. However, the need to better understand the biological phenomena related to the variations resulting from different feeding frequencies, nutritional composition and proportion of diet ingredients, and the physiological status of the animal are necessary to be able to determine the best feeding strategies to obtain better productive performances and better quality products.

Feeding frequency did not influence the content of blood metabolites or liver enzyme activity in lambs. All levels were within the reference values observed in sheep, urea (32.0–66.0 mg/dL), TP (4.5–8.2 g/dL), GGT (52.2–67.0), and AST (68.0–168.0) [64–69]. This indicates that the change in the frequency of feeding did not affect the normal functioning of the liver or the transport of blood metabolites.

5. Conclusions

Based on the results from this study, it is possible to conclude that the feeding frequency of up to four times does not affect the productive performance, carcass characteristics, or meat quality of feedlot male lambs. From this, it is possible to recommend the adoption of a feeding frequency of once a day.

The feeding frequency of more than once a day promoted greater digestibility of NDF, total digestible nutrients, and subcutaneous fat thickness; also, these feeding frequencies decreased the rumination time of the lambs.

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References
1. Alba, H.; Júnior, J.F.; Leite, L.; Azevêdo, J.; Santos, S.; Pina, D.; Cirne, L.; Rodrigues, C.; Silva, W.; Lima, V.; et al. Protected or Unprotected Fat Addition for Feedlot Lambs: Feeding Behavior, Carcass Traits, and Meat Quality. *Animals* 2021, 11, 328. [CrossRef]
2. De Oliveira, C.R.N.; Santos, S.A.; Mariz, L.D.S.; Carvalho, G.G.P.; de Azevêdo, J.A.G.; Tosto, M.S.L.; dos Santos, A.C.S. Dietary phase-feeding as feedlot strategy for Santa Inês lambs: Performance, N retention and meat quality. *Livest. Sci.* 2020, 239, 104106. [CrossRef]
3. Nascimento, C.; Pina, D.; Cirne, L.; Santos, S.; Araújo, M.; Rodrigues, T.; Silva, W.; Souza, M.; Alba, H.; de Carvalho, G. Effects of Whole Corn Germ, A Source of Linoleic Acid, on Carcass Characteristics and Meat Quality of Feedlot Lambs. *Animals* 2021, 11, 267. [CrossRef]
4. Chikwanha, O.; Muchenje, V.; Nolle, J.E.; Dugan, M.E.; Mapieyi, C. Grape pomace (*Vitis vinifera* L. cv. Pinotage) supplementation in lamb diets: Effects on growth performance, carcass and meat quality. *Meat Sci.* 2018, 147, 6–12. [CrossRef]
5. Guerreiro, O.; Alves, S.P.; Soldado, D.; Cachuchu, L.; Almeida, J.M.; Francisco, A.; Santos-Silva, J.; Bessa, R.J.; Jerónimo, E. Inclusion of the aerial part and condensed tannin extract from *Cistus ladanifer* L. in lamb diets—Effects on growth performance, carcass and meat quality and fatty acid composition of intramuscular and subcutaneous fat. *Meat Sci.* 2019, 160, 107945. [CrossRef]
6. Valença, R.D.L.; Sobrinho, A.G.D.S.; Borghi, T.H.; Meza, D.A.R.; de Andrade, N.; Silva, L.G.; Bezzerra, L.R. Performance, carcass traits, physicochemical properties and fatty acids composition of lamb’s meat fed diets with marine microalgae meal (*Schizochytrium sp.*). *Livest. Sci.* 2020, 243, 104387. [CrossRef]
7. Hart, K.; McBride, B.; Duffield, T.; Devries, T. Effect of frequency of feed delivery on the behavior and productivity of lactating dairy cows. *J. Dairy Sci.* 2014, 97, 1713–1724. [CrossRef] [PubMed]
8. Judy, J.; Bachman, G.; Brown-Brandl, T.; Fernando, S.; Hales, K.; Miller, P.; Stowell, R.; Kononoff, P. Energy balance and diurnal variation in methane production as affected by feeding frequency in Jersey cows in late lactation. *J. Dairy Sci.* 2018, 101, 10899–10910. [CrossRef]
9. Benchaar, C.; Hassanat, F. Frequency of diet delivery to dairy cows: Effect on nutrient digestion, rumen fermentation, methane production, nitrogen utilization, and milk production. *J. Dairy Sci.* 2020, 103, 7094–7109. [CrossRef] [PubMed]
10. Dias, M.S.d.S.; Ghizzi, L.G.; Marques, J.A.; Nunes, A.T.; Grigoletto, N.T.; Gheller, L.S.; Silva, T.B.; Silva, G.G.; Lobato, D.N.; e Silva, L.F.C.; et al. Effects of organic acids in total mixed ration and feeding frequency on productive performance of dairy cows. *J. Dairy Sci.* 2021, 104, 5405–5416. [CrossRef]
11. Bunting, L.D.; Howard, M.D.; Muntifering, R.B.; Dawson, K.A.; Boling, J.A. Effect of Feeding Frequency on Forage Fiber and Nitrogen Utilization in Sheep. *J. Anim. Sci.* 1987, 64, 1170–1177. [CrossRef] [PubMed]
12. Carter, R.R.; Allen, O.B.; Grovum, W.L. The effect of feeding frequency and meal size on amounts of total and parotid saliva secreted by sheep. *Br. J. Nutr.* 1990, 63, 305–318. [CrossRef]
13. Abouheiç, M.; Alshamiry, F.; El-Waziry, A.; Ali, M.; Swelum, A. Effect of Feeding Frequency on Plasma Metabolites Concentrations and Production Cost in Feed-restricted Lambs. *Anim. Nutr. Feed. Technol.* 2017, 17, 279. [CrossRef]
14. Sun, X.; Chen, A.; Pacheco, D.; Hoskin, S.O.; Luo, D. Sheep Rumen Fermentation Characteristics Affected by Feeding Frequency and Feeding Level When Fed Fresh Forage. *Animals* 2019, 10, 7. [CrossRef]
15. Macmillan, K.; Gao, X.; Oba, M. Increased feeding frequency increased milk fat yield and may reduce the severity of subacute ruminal acidosis in higher-risk cows. *J. Dairy Sci.* 2017, 100, 1045–1054. [CrossRef] [PubMed]
16. Hegarty, R.S. Genotype differences and their impact on digestive tract function of ruminants: A review. *Aust. J. Exp. Agric.* 2004, 44, 459. [CrossRef]
17. Claus, M.; Hummel, J. Physiological adaptations of ruminants and their potential relevance for production systems. *Rev. Bras. Zootec.* 2017, 46, 606–613. [CrossRef]
18. Amorim, S.T.; Kluksa, S.; Berton, M.P.; De Lemos, M.V.A.; Peripolli, E.; Stafuzzza, N.B.; Martin, J.F.; Álvarez, M.S.; Gaviña, B.V.; Toró, M.A.; et al. Genomic study for maternal related traits in Santa Inês sheep breed. *Livest. Sci.* 2018, 217, 76–84. [CrossRef]
19. De Sousa, W.H.; Cartaxo, F.Q.; Costa, R.G.; Cezar, M.F.; Cunha, M.D.G.G.; Filho, J.M.P.; Dos Santos, N.M. Biological and economic performance of feedlot lambs fed diets with different energy densities. *Rev. Bras. de Zootec.* 2012, 41, 1285–1291. [CrossRef]
20. Juca, A.; Favari, J.C.; Filho, G.M.M.; Filho, A.D.L.R.; Azevedo, H.C.; Muniz, E.N.; Pinto, L.F.B. Performance of the Santa Inês breed raised on pasture in semiarid tropical regions and factors that explain trait variation. *Trop. Anim. Health Prod.* 2014, 46, 1249–1256. [CrossRef]
21. Gallo, S.B.; Arrigoni, M.; Lemos, A.L.D.S.C.; Haguivara, M.M.H.; Bezzerra, H. Influence of lamb finishing system on animal performance and meat quality. *Acta Sci. Anim. Sci.* 2018, 41, 44742. [CrossRef]
47. Weiss, C.P.; Gentry, W.W.; Meredith, C.M.; Meyer, B.E.; Cole, N.A.; Tedeschi, L.O.; Mccollum, F.T.; Jennings, J.S. Effects of roughage inclusion and particle size on digestion and ruminal fermentation characteristics of beef steers. J. Anim. Sci. 2017, 95, 1707–1714. [CrossRef] [PubMed]

48. Dijkstra, J.; Ellis, J.; Kebrab, E.; Strathe, A.; Lopez, S.; France, J.; Bannink, A. Ruminal pH regulation and nutritional consequences of low pH. Anim. Feed. Sci. Technol. 2012, 172, 22–33. [CrossRef]

49. Chibisa, G.E.; Beauchemin, K.A.; Penner, G.B. Relative contribution of ruminal buffering systems to pH regulation in feedlot cattle fed either low- or high-forage diets. Animals 2016, 10, 1164–1172. [CrossRef]

50. Castillo-Lopez, E.; Petri, R.M.; Ricci, S.; Rivera-Chacon, R.; Sener-Aydemir, A.; Sharma, S.; Reisinger, N.; Zebeli, Q. Dynamic changes in salivation, salivary composition, and rumen fermentation associated with duration of high-fodder feeding in cows. J. Dairy Sci. 2021, 104, 4875–4892. [CrossRef]

51. Bach, A.; Calsamiglia, S.; Stern, M. Nitrogen Metabolism in the Rumen. J. Dairy Sci. 2005, 88, E9–E21. [CrossRef]

52. Huang, Y.; Zhou, F.; Nan, Z.; Zhou, N. Comparative Grain Yield, Straw Yield, Chemical Composition, Carbohydrate and Protein Fractions, In Vitro Digestibility and Rumen Degradability of Four Common Vetch Varieties Grown on the Qinghai-Tibetan Plateau. Animals 2019, 9, 505. [CrossRef] [PubMed]

53. Goulart, R.S.; Vieira, R.; Daniel, J.L.P.; Amaral, R.C.; Santos, V.P.; Cabezas-Garcia, E.H.; Tedeschi, L.; Nussio, L.G. Effects of source and concentration of neutral detergent fiber from roughage in beef cattle diets on feed intake, ingestive behavior, and ruminal kinetics. J. Anim. Sci. 2020, 98, skaa107. [CrossRef]

54. Beltrán-Fernández, E.; Silva, A.M.D.A.; Filho, J.M.P.; De Moura, J.F.P.; De Oliveira, J.P.F.; Oliveira, R.L.; Dias-Silva, T.P.; Bezerra, L.R. Effect of different blend levels of spineless cactus and Mombasa hay as roughage on intake, digestibility, ingestive behavior, and performance of lambs. Trop. Anim. Health Prod. 2021, 53, 1–7. [CrossRef]

55. Filho, A.E.; de Carvalho, G.G.P.; Pires, A.J.V.; Silva, R.R.; Santos, P.E.F.; Murta, R.M.; Pereira, F.M. Ingestive Behavior of Lambs Confined in Individual and Group Stalls. Asian-Australasian J. Anim. Sci. 2014, 27, 284–289. [CrossRef]

56. Van, D.T.T.; Mui, N.T.; Ledin, I.; Mui, N.T. Effect of group size on feed intake, aggressive behaviour and growth rate in goat kids and lambs. Small Rumin. Res. 2007, 72, 187–196. [CrossRef]

57. Aleme, H.; Getabalew, M. Factors influencing the growth and development of meat animals. Int. J. Anim. Sci. 2019, 3, 1048. [CrossRef]

58. Hocquette, J.-F. Endocrine and metabolic regulation of muscle growth and body composition in cattle. Animals 2010, 4, 1797–1809. [CrossRef]

59. Zhao, L.; Huang, Y.; Du, M. Farm animals for studying muscle development and metabolism: Dual purposes for animal production and human health. Anim. Front. 2019, 9, 21–27. [CrossRef]

60. Urrutia, N.; Zhou, F.; Nan, Z.; Zhou, N. Comparative Grain Yield, Straw Yield, Chemical Composition, Carbohydrate and Protein Fractions, In Vitro Digestibility and Rumen Degradability of Four Common Vetch Varieties Grown on the Qinghai-Tibetan Plateau. Animals 2019, 9, 505. [CrossRef] [PubMed]

61. Bionaz, M.; Vargas-Bello-Pérez, E.; Busato, S. Advances in fatty acids nutrition in dairy cows: From gut to cells and effects on performance. J. Anim. Sci. Biotechnol. 2020, 11, 1–36. [CrossRef]

62. Dehority, B.A.; Tirabasso, P.A. Effect of feeding frequency on bacterial and fungal concentrations, pH, and other parameters in the rumen. J. Anim. Sci. 2001, 79, 2908–2912. [CrossRef]

63. Da Silva, J.; Carrara, T.V.B.; Pereira, M.; De Oliveira, C.A.; Júnior, I.C.B.; Watanabe, D.H.M.; Rigueiro, A.L.N.; Arrigoni, M.; Millen, D.D. Feedlot performance, feeding behavior and rumen morphometrics of Nellore cattle submitted to different feeding frequencies. Sci. Agricola 2018, 75, 121–128. [CrossRef]

64. Przemyslaw, S.; Cezary, P.; Stanislaw, M.; Krzysztof, L.; Barbara, P.; Zofia, A.; Maja, F.; Katarzyna, Ź.; Ząbek, K. The effect of nutritional and fermentational characteristics of grass and legume silages on feed intake, growth performance and blood indices of lambs. Small Rumin. Res. 2015, 123, 1–7. [CrossRef]

65. Gobindram, M.N.N.-E.; Bognanno, M.; Luciano, G.; Lanza, M.; Biondi, L. Carob pulp inclusion in lamb diets: Effect on intake, performance, feeding behaviour and blood metabolites. Anim. Prod. Sci. 2016, 56, 850–858. [CrossRef]

66. Shakeri, P. Pistachio by-product as an alternative forage source for male lambs: Effects on performance, blood metabolites, and urine characteristics. Anim. Feed. Sci. Technol. 2015, 211, 92–99. [CrossRef]

67. Costa, R.G.; Ribeiro, N.L.; Nobre, P.T.; Carvalho, F.; Medeiros, A.N.; Cruz, G.R.B.; Freire, L.F.S. Biochemical and hormonal parameters of lambs using guava (Psidium guajava L.) agro-industrial waste in the diet. Trop. Anim. Health Prod. 2017, 50, 217–221. [CrossRef] [PubMed]

68. Odhaib, K.J.; Adeyemi, K.; Ahmed, M.A.; Jahromi, M.F.; Jusoh, S.; Samsudin, A.A.; Alimon, A.R.; Yaakub, H.; Sazili, A.Q. Influence of Nigella sativa seeds, Rosmarinus officinalis leaves and their combination on growth performance, immune response and rumen metabolism in Dorper lambs. Trop. Anim. Health Prod. 2018, 50, 1011–1023. [CrossRef] [PubMed]

69. Rodrigues, T.C.G.D.C.; Freitas, P.M.; Santos, E.M.; De Araújo, G.G.L.; Pires, A.J.V.; Ayres, M.C.C.; De Carvalho, L.M.; Souza, J.; De Carvalho, G.G.P. Effects of ammoniated pearl millet silage on intake, feeding behavior, and blood metabolites in feedlot lambs. Trop. Anim. Health Prod. 2019, 51, 2323–2331. [CrossRef] [PubMed]