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The effect of feed processing on ruminal parameters in intensively fattened lambs

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Abstract

The aim of the study was to assess the influence of three processing ways of the concentrate feed on ruminal dry matter degradability, ruminal pH and ruminal protozoa population. Pelleted concentrate feed was mechanically processed to a particle size of ø 5 mm (variant A), ground at ø 2 mm (variant B) or by replacing the 50% of ground corn grain with expanded corn with ø 2 mm particle size as in B (variant C). The trial was carried out alternately on three lambs during their 90-132 days of life fitted with ruminal fistula. Feed bags and ruminal fluid was collected through the ruminal cannula at 3, 6, 9, 12, and 24 hours of incubation. After 24 hours of incubation the intra-ruminal feed concentrate dry matter degradation was significantly higher in expanded concentrate compared to pelleted concentrate (80.81% vs. 70.48%, P<0.049). The same parameter had an intermediary value of 77.85% for ground feed concentrate. Reducing pH value of the ruminal fluid from values of over 5.7 to an average of 5.3 resulted in reduction of the protozoa number per ml of ruminal fluid from 8.859 x 10^5 to 4.208 x 10^5. The lowest value for protozoa was obtained in variant A, 1.953 x 10^4 protozoa/ml ruminal fluid, at a pH of 5.10 (following 6 hours of fermentation). It was concluded that out of all the identified protozoa, the Entodinium sp. was the dominant genus, making up 98.20% to 100% of the ruminal protozoa.

Key words: lambs, feed processing, concentrate feed degradability, pH, protozoa

1. Introduction

In the intensive fattening system for lambs, forage mixtures with the size of 3 to 5 mm are being used. Granulation provides compaction of various components that were previously grounded [1]. Studies have shown that any change of the feed source, by mechanical processing of the cereal grain, which reduces the particle size determines pH reduction of the ruminal fluid in ruminants [2]. Mechanical processing of the cereals influences the salivary glands secretion and thus the carbohydrates degradation at the rumen level is affected. Thus, a negative relationship could be established between the carbohydrates metabolizing capacity, and intra-ruminal digestibility rate on one hand and pH of the ruminal liquid on the other hand [2, 3].

Feed digestibility and pH could be influenced by a large number of factors, one of them being the chemical composition of the feed [4]. Introducing hay in the finishing lambs diet increases the ruminal pH, but affects the digestibility, nitrogen retention, mastication and ruminal parameters as well [5].

Chemical composition of the pellets can be altered by supplementation with nutrients that might have an effect on the microbial populations. Addition of fibrolytic enzymes (12 g/d;

ENZ) increased the digestibility, ciliate protozoa activity and microbial protein synthesis. These products have an important function in determining the increase of the fibrolytic activity and development of the cellulosolitic bacteria with function in feed decomposition and a higher digestibility [6]. Feeding magnesium enriched additives quantitatively and qualitatively modifies the ruminal ciliate protozoa and implicitly the fermentation process at the rumen level [7].

Ruminal fluid collecting procedure could have an influence on ruminal fluid pH value. Thus, Garrett et al. [8] determined that if ruminal fluid is obtained by rumenocentesis the pH value would be 0.28 units lower compared to obtaining it through a ruminal cannula. Ruminal fluid filtration or aspiration had no effect on the pH value.

Maintaining pH value within certain limits is absolutely necessary taking into account the pH influence on the rumen protozoa communities' development that have an important role on digestion and digestibility. Thus, maintaining the pH between 5.5 and 7.5 is influenced by the diet and the feeding frequency according to different ruminant species [9].

Protozoa number decrease could be influenced by the feeding supplements. Thus, adding sunflower oil in the diet determines decrease of the total protozoa count in the ruminal fluid and maintains the protozoa count at a low level for up to 6 days [10].

Ruminal ciliate protozoa proved to be sensitive to variations of the ruminal fluid pH value and cannot survive to pH higher than 7.8 and lower than 5. Dehority [11] demonstrated that, *in vitro,* the majority of the protozoa die at pH values lower than 5.4.

Variation of protozoa count in the rumen fluid has an important function, being an indicator for acute and subacute acidosis determined by the lactic acid relative to the volatile fatty acids in rumen. Thus, protozoa count in rumen could be used to diagnose the ruminal acidosis [12].

Absence of ciliate protozoa in lambs' rumen could have negative effects on ruminal microbial ecology, feed sources biodegradation processes and muscle fatty acids composition [13]. Ciliate protozoa absence could determine a decrease of the nutrient digestibility and an increase in ruminal TVFA and total-N with lower NH3-N concentration, indicating better energy and protein utilization in defaunated lambs [14].

The aim of the study was to establish the influence of concentrate feed processing on the dry matter degradability rate, rumen pH, and ruminal protozoa population.

2. Materials and methods

Animals, Diets

The study was carried out on three weaned Turcana lambs, with similar weights (start up 26.5 kg) obtained from single lambing. Microclimate factors were maintained within the physiological requirements as follows: 12-15°C average air temperature, 70-75% relative humidity, 0.2 m/s air speed, maximum 0.03% CO_2 concentration, and maximum 0.002% NH_3 concentration [15].

Lambs were surgically fitted with rumen cannula in the veterinarian clinic by qualified personnel having the Ethical Commission approval according to the European Union`s Directive (2010)/63/EU for animal experimentation [16].

Lambs were fed alfalfa hay at 300 g/head/day and concentrate feed *ad libitum* as follows: A) concentrate feed pelleted at ø 5 mm; B) the same concentrate feed ground at ø 2 mm; and C) a concentrate feed ground at ø 2 mm, in which 50% of the corn was replaced by expanded corn.

In our study were used same three lambs for each concentrate feed type. The assessment was carried out after 12 days of transition to the new feed type and restoring the protozoa communities balance. The entire study's time length was of 42 days (12 day adaptation + 2 days sample collection x 3 experimental periods).

Intra-ruminal dry matter degradability assessment

Concentrate feed was weighed using Kern ALJ220-4NM analytic balance and was introduced into the lamb's rumen through the ruminal fistula in five bags per lamb, for the following time intervals of incubation: 3, 6, 9, 12, and 24 hours. The effective degradability of DM was estimated by incubating nylon bags with a pore size of 50 μm assuring a ratio of 15.6 mg DM/cm^2 of bag surface area, method described by Orskov [17]. Undigested fractions were dried in the Memmert UNB500 oven at 60°C for 2-3 hours followed by drying at 105 for 2-3 hours in the subsequent day. Weight was recorded after ensuring complete drying in the second day. Difference in the dry matter in the sample before and after incubation in rumen was considered as intra-ruminal degradability.

Determining the ruminal fluid pH

Ruminal liquid analysis was carried out at the same time with the degradability, when the bags were taken out through the ruminal cannula by using a sterile hose and test tubes. Zero incubation time was considered the time when the bags were introduced into the rumen. pH was determined at every ruminal fluid collection. Ruminal fluid pH was determined by using the multi-parameter digital device Multimetru WTW 340i/SET.

Ruminal fluid protozoa count and genera determination

Infusoria number determination consisted in assessment of the infusoria number per volume unit (mm^3) after immobilisation with a 1% formaldehyde solution, method described by Dehority [18]. Materials and reagents used were: 1% formaldehyde solution; Fuchs-Rosenthal haemocytometer, cover glasses, pipettes, and Optika B100 microscope. One millilitre ruminal fluid was treated with 4 ml 1% formaldehyde solution. The lamina was applied by pressure to haemocytometer until the Newton circles appeared. With the Pasteur pipette 1-2 ruminal fluid drops were applied at the edge of the lamina, which by capillarity penetrated under the lamina. It was kept undisturbed for 2-3 minutes in order for the infusoria to distribute and stabilize within the haemocytometer grid. After that the infusoria were counted in 100 microscopic fields at 100x magnitude. Calculation was carried out using the following formula: $N = \frac{n\,x\,5\,x\,1000}{3.2}$

where: N – infusoria number per mm^3; n – number of counted infusoria; 5 - dilution; 3.2 counting camera volume; 1000 – correction coefficient.

The main ruminal infusoria genera were determined taking into account that they belong to subclass Ciliata, are about 30 genera, and are grouped in large, medium, and small categories according to their size (20-200 μm).

Statistical analysis

Data was analysed using variance analysis, simple correlation as the 2nd degree polynomial regression equations. The software employed was MIMITAB 14. Variance analysis was based on the following equations: $X = \frac{\sum x}{n}$; $Sx^2 = \Sigma x^2 - TC$; $TC = \frac{(\sum x)^2}{n}$; $S^2 = \frac{Sx^2}{GL}$; $SD = \sqrt{S^2}$; $SEM = \frac{S}{\sqrt{n}}$; $CV\% = \frac{S \cdot 100}{X}$; where: X = average around which the variable values are grouping; Sx^2 = squared deviations sum; n = individuals number; Σx =

individuals sum; TC = correlation term; S^2 = variance; SD = standard deviation; SEM = standard error of the mean; CV % = variability coefficient; GL = n-1 (liberty degrees).

Significant difference in variables was tested using Mann-Whitney u test at 0.05 level of probability.

3. Results and Discussions

Evaluation of concentrate feed degradability

Lambs fed with pelleted concentrate feed had moderately acidic pH ranged from 5.10 to 5.34. Feed DM degradability gradually increased to an average of 70.48% at 24 hours of incubation with a range of 67.05% to 74.65% (Table 1).

Table 1 Dry matter digestive use coefficient of the concentrate feed (%)

Item	Pelleted concentrate feed at ø 5mm (variant A) n=3			Ground concentrate feed at ø 2mm (variant B) n=3			Ground concentrate feed at ø 2mm (50 % expanded corn) (variant C) n=3		
Incubation hours	X±SEM	SD	pH	X±SEM	SD	pH	X±SEM	SD	pH
3	34.35±0.67	1.16	5.34	39.72±2.09	3.61	5.34	38.45±1.10	1.90	5.28
6	42.28±3.60	6.23	5.10	44.60±1.73	2.99	5.25	52.41±3.39	5.87	5.58
9	49.92±1.48	2.56	5.11	51.30±1.31	2.27	5.64	57.36±2.56	4.42	5.38
12	55.25±3.30	5.72	5.10	58.59±1.92	3.33	5.52	62.89±1.39	2.40	5.36
24	70.48±2.23	3.85	5.30	77.85[a]±4.60	7.96	6.08	80.51[b]±1.91	3.31	5.73

a – variant A vs. variant B; P>0.275

b - variant A vs. variant C; P<0.049

When the concentrate feed given to lambs was ground at 2 mm, ruminal fluid pH ranged within larger limits from 5.25 to 6.08. DM degradability coefficient varied within larger limits, as well. After 24 hours of incubation the concentrate feed degradability took values between 60.10% and 84.65%, with an average of 77.85%.

Using expanded corn in the concentrate feed structure ground at 2 mm, ruminal fluid pH ranged from 5.28 to 5.73, and increased the DM degradability, after 24 hours of incubation, to an average of 80.51%, with limits from 77.20% to 83.81%.

The second degree polynomial regression was used to model the evolution of the intra-ruminal bag degradability coefficient of the pelleted and ground concentrate feed (Figure 1).

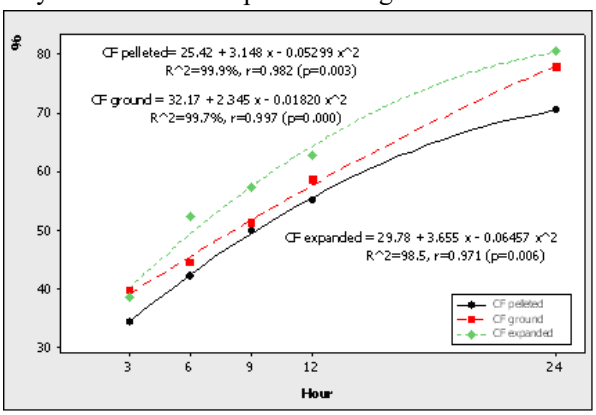

Figure 1. Graphical representation of the digestive use index for the concentrate feed processed in various ways, in relation to time (y), modelled with the help of second degree polynomial regression

Positive correlation between feed degradability coefficient and incubation time (0 to 24 hours) was observed with a significant r value, as follows: 0.982 in variant A, 0.997 in variant B, and 0.971 in variant C.

The highest degradability after 3 hours of intra-ruminal incubation was obtained in variant B, and after 6, 9, 12, and 24 hours in variant C. The lowest values of the intra-ruminal digestion were obtained in variant A.

After 24 hours of intra-ruminal incubation the pelleted concentrate feed is digested by 10.45 percentage less than ground concentrate feed and by 14.23 percentage ($P<0.049$) less than concentrate feed containing 50% expanded corn.

Protozoa in ruminal fluid evaluation

Ruminal infusoria load is strictly dependent of the microbial population size and the presence of particular nutrients ingested by the lamb, which is represented by the type of processing the concentrate feed: pelleting, grounding or expanding (Table 2; Figure 2).

Table 2 Number and type of the protozoa isolated from the ruminal fluid in lambs

Item	Pelleted concentrate feed at ø 5 mm (variant A) n=3		Ground concentrate feed at ø 2 mm (variant B) n=3		Ground concentrate feed at ø 2 mm with 50% expanded corn (variant C) n=3	
Incubation hours	Average Nr /ml	Genus	Average Nr /ml	Genus	Average Nr /ml	Genus
0	5.859 x 10^5	*Entodinium* 100 %	3.226 x 10^5	*Entodinium* 98.62 % *Dasytricha* 1.38 %	6.812 x 10^5	*Entodinium* 99.13 % *Dasytricha* 0.87 %
3	2.966 x 10^4	*Entodinium* 100 %	1.216 x 10^5	*Entodinium* 99.44 % *Dasytricha* 0.56 %	5.257 x 10^5	*Entodinium* 99.16 % *Dasytricha* 0.84 %
6	1.953 x 10^4	*Entodinium* 100 %	2.670 x 10^5	*Entodinium* 99.37 % *Dasytricha* 0.63 %	7.421 x 10^5	*Entodinium* 99.41 % *Dasytricha* 0.59 %
9	4.530 x 10^4	*Entodinium* 100 %	2.812 x 10^5	*Entodinium* 99.69 % *Dasytricha* 0.31 %	6.218 x 10^5	*Entodinium* 99.22 % *Dasytricha* 0.78 %
12	6.612 x 10^4	*Entodinium* 100 %	8.177 x 10^5	*Entodinium* 100 %	8.606 x 10^5	*Entodinium* 98.20 % *Dasytricha* 1.80 %
24	4.208 x 10^5	*Entodinium* 100 %	5.737 x 10^5	*Entodinium* 100 %	8.859 x 10^5	*Entodinium* 99.40 % *Dasytricha* 0.60 %

Pelleted concentrate feed reduced the protozoa count in the first 6 hours of incubation (1.953 x 10^4/mL). However, this increased to an average of 4.208 x 10^5/mL at 24 hours of incubation. Protozoa identified in ruminal fluid was belonged absolutely to *Entodinium* genus and no other genus was observed in variant A.

When the concentrate feed was ground at 2 mm, the infusoria count followed the same trend of decreasing during the first 6 hours of incubation after feeding and increased to a peak

at 12 hours to an average of 8.177 x 10^5 mL. After 24 hours of incubation the protozoa count decreased at an average of 5.737 x 10^5/mL. Protozoa identified in ruminal fluid was belonged 100% to *Entodinium* genus, except for samples collected at 9 hours of incubation, when a small proportion (0.31% up to 1.38%) of the protozoa belonged to *Dasytricha* genus.

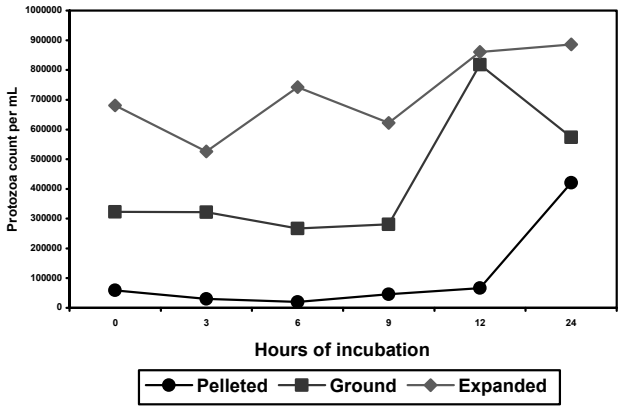

Figure 2. Graphical representation of the protozoa count isolated from the ruminal fluid in lambs fed with concentrate feed in various processing forms

Using expanded corn in the concentrate feed formulation, ground at 2 mm, determined a decrease in protozoa count during the first 3 hours of incubation, followed by an increase at 6 hours, then a slight decrease at 9 hours followed by another increase reaching an average of 8.859 x 10^5 protozoa/mL ruminal fluid at 24 hours of incubation. Protozoa diversity was higher in variant C. Thus, whereas the *Entodinium* genus was predominant, a proportion of 0.6% to 1.8% of *Dasytricha* genus protozoa was identified during the whole 24 hours incubation time.

These results lead us to the observation that after 24 hours of incubation, the diversity and number of infusors identified from the ruminal fluid, being 2.1 times higher in variant C compared to variant A and 1.54 times higher compared to variant B. Also, the protozoa count is 1.36 times lower in variant A compared to variant B, at the same incubation time.

Ruminal pH value has an important function regarding the concentrate feed dry matter digestibility, and protozoa (ciliate) survival and development. Rumen pH was below 5.73 in any processed feed fed to lambs. Dehority [18] reported decreased ruminal pH after feeding processed feeds. In general soon after ingestion of diet pH would fall but, raises in the postprandial intervals [12] as evident from increased pH between 6 to 12 h in this study. In the subsequent intervals also rumen pH was increased to 6.08 and 5.73 on variant B and variant C but not on variant A.

In our study we have observed that decreasing pH value lead to decrease in protozoa count per millilitre of ruminal fluid and also to a decreased intra-ruminal degradability of the concentrate feed. Similar results have been reported by other researchers who studied the effect of ruminal pH on digestibility [19]. Infusors-load is strictly dependent on the size of microbial populations and the presence of certain nutrients ingested by the lamb. Also, it may be observed within the same experimental group, high differences between the protozoa count from the ruminal fluid samples collected.

Changing the pH value according to the feed and processing way of the feed was noticed in other studies as well [18]. The pH value varies greatly according to the time of feeding, thus, after feeding pH increases at interval between 8 to 12 h [12]. Values of pH determined in the ruminal fluid collected from lambs, during the time interval from zero (time of feeding) until 24 hours after feeding confirmed the existence of a variation in ruminal pH.

Significantly high correlation between the ruminal protozoa count and ruminal pH was observed. Drastic decrease in the ruminal protozoa count in lambs leads to decreased ruminal ammonia concentration and pH value [14].

Maintaining the ruminal fluid pH at a low level during the day determines a reduction of the ruminal ciliate protozoa up to total extinction. It looks like animals have a very efficient controlling mechanism of the pH level, and thus the ruminal pH controls have a significant influence on the ruminal ciliate protozoa population [20].

Although pH values below 6.08 had negative influence on the ruminal protozoa community, in our study, unaffected population consisted 98.2 to 100% of *Entodinium* and just 0.31 to 1.80 % of *Dasytricha* genus. These results are concordant with the inferences drawn by Lyle et al. [21], about the tolerance of acidic pH by *Entodinium* species. However, Dehority [11] stated that there are no scientific proofs to explain the acidic tolerance of species from the *Entodinium* genus that is 98% of the total protozoa population in rumen. It is supposed that these species presence has been given by a change without a clear cause of the intra-ruminal conditions. Using classical identification methods of the ruminal protozoa, Ajisaka et al. [22] quantified the existence of *Entodinium* sp., in rumen was 10^5/mL while other genera like *Dasytricha* sp., and, *Isotricha* sp., was 10^3/mL.

Although according to Skillman et al.[23], protozoa number is relatively constant in rumen, great difference exit among type of protozoa. In the present study, the ciliate protozoa identified in the rumen belonged to *Entodinium sp.* and *Dasytricha sp.* and the number of protozoa was about 10^4-10^5/ mL and 10^3-10^4/ mL respectively.

Entodinium and *Dasytricha* genus are very phylogenetically related. Cluster type statistical studies based on modern identification techniques allowed to establish this relationship [24].

Analyzing the results obtained in the specific operating conditions, including biological material belonging to an old native breeds, we can say that our data complete the vast previous researches done on similar topics.

4. Conclusion

Processing the concentrate feed components at size of 5 mm (variant A) and 2 mm (variant B) had no significant influence ($P>0.275$) on feed degradability. Replacing 50% of corn with expanded corn in concentrate feed composition ground at 2 mm (variant C) significantly increased ($P<0.049$) the intra-ruminal degradability compared to variant A.

Reducing the pH below the value of 5.2 decreased up to 10 times the protozoa count of the ruminal fluid.

The protozoa count from the ruminal fluid was lower in variable proportions during 24 hours period in variant A compared to variant B. Expanding the corn added in the concentrate feed increased both the protozoa count and genus diversity. *Entodinium* genus was the predominant genus in all the ruminal fluid samples collected.

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