

Keratinisation degree of rumen epithelium and body condition score in sheep grazing on *Brachypodium rupestre*

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Keywords

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Sheep.

Summary

This article describes the result of a study focusing on the keratinisation degree of rumen mucosa and changes in the body condition of sheep that had grazed for 20 days in a pasture densely covered with *Brachypodium rupestre*. Grazing in this type of pasture can reduce the probability of fires in a Mediterranean mountain setting. However, foraging in areas with a prevalence of *Brachypodium rupestre* can affect animals' well being. In this respect, it is essential to determine the length of time during which the animals can remain in this environment before their welfare is compromised by this type of pasture. Ewes grazing on a semi-mesophilic pasture were included as a control. On days 1, 10, and 20, five ewes from each group were sacrificed to evaluate the variations of the epithelial keratinization degree of the rumen atrium and ventral sac. Body weight (BW) and body condition score (BCS) were assessed in ten ewes per group. The control animals showed little variation in the keratinisation degree of rumen mucosa without any detrimental effects on the BCS and BW. The experimental animals showed a significant increase in the epithelial keratinisation degree within 10 days and a decrease of BCS and BW within 20 days. The data collected suggest that animals should not remain for longer than 10-12 days on pasture highly covered with *Brachypodium rupestre*.

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Introduction

The European Union, besides promoting and defending animal welfare, supports research focused on the use of domestic animals to prevent forest fires (Rule CE 1782/2003). Necromass on the periphery of pastures, which are often characterised by a dense covering of *Brachypodium rupestre*, is a potential cause of forest fires. This tall grass is unfavourable for ovine grazing because it is rich in silicates and has highly fibrous leaves (17). However, sheep that are fenced in areas covered with *Brachypodium rupestre* tend to exploit all foraging resources and may remove the fire primer.

When *Brachypodium* is not grazed, it tends to spread on pastures which limits the growth of more palatable species weakening the sexual and vegetative reproduction and spread phases of these species (4, 10, 12, 19). This affects the floristic composition of the pasture, which can in turn lead to an ecological imbalance that lowers grazing value and biodiversity (1, 2, 7). The primary pastures in the Italian Apennine

Mountains are located within sites of community interest and special protection areas (8). Therefore, it is necessary to limit the dispersal of highly invasive species that can negatively affect the biodiversity in these territories.

Sheep breeders who allow their flocks to graze in areas that are at an increased risk of fires could use public funds for fire prevention and increase the aid of husbandry through the Common Agricultural Policy (9), which is linked to the use of the best practices aimed at preserving biodiversity and respecting animal welfare.

This study evaluated the degree of keratinisation in the ruminal mucosa using optical microscopy and changes in the body condition based on trends in mean body weight (BW) and body condition score (BCS) of sheep grazing on *Brachypodium rupestre*. The purpose of the analysis was to determine the length of time the animals can remain in these pastures without their welfare being negatively affected.

Materials and Methods

Animals and experimental plan

The experiments were performed during the month of October 2009. A total of 50 Comisana x Appenninica yearling sheep were used. All the animals were nulliparous and housed without rams. Twenty five animals (experimental group) were maintained for 4 days on a plot with sparse coverage (30%) of *Brachypodium rupestre*. The animals were then transferred to a densely (60%) covered plot for 20 days (from day 1 to day 20). Twenty five sheep in the same physiological conditions as the experimental animals were maintained in a semi-mesophilic natural pasture (control group) (5). In both groups, 10 sheep were used for BCS and BW evaluations, while five sheep were sacrificed at the beginning of the experiment and 10 and 20 days after grazing on respective pasture (day 1, 10, and 20).

Routine controls to monitor the health of the animals, including direct macroscopic observations of the lip, tongue and buccal mucosae to screen for

the presence of macrolesions, were performed on all the living animals.

Morphological evaluation of the rumen mucosa

Animals in the experimental and control groups were slaughtered at the Visso (Macerata, Italy) slaughterhouse. Samples from the rumen atrium and ventral sac wall were removed, fixed in Bouin's fluid and embedded in paraffin wax.

Morphological evaluations to assess the keratinised layer were performed on 5- μ m thick tissue sections using the following stains: haematoxylin and eosin, Mallory's trichrome, and Floxin B/Orange G/Alcian blue (16). To avoid variations in staining due to slight differences in temperature or incubation, all the samples were processed concurrently during each step of the procedure.

To evaluate changes or damage to the mucosa of the rumen caused by *Brachypodium rupestre*, the tissue sections were observed under a light microscope (Eclipse E800, Nikon Corporation, Tokyo,

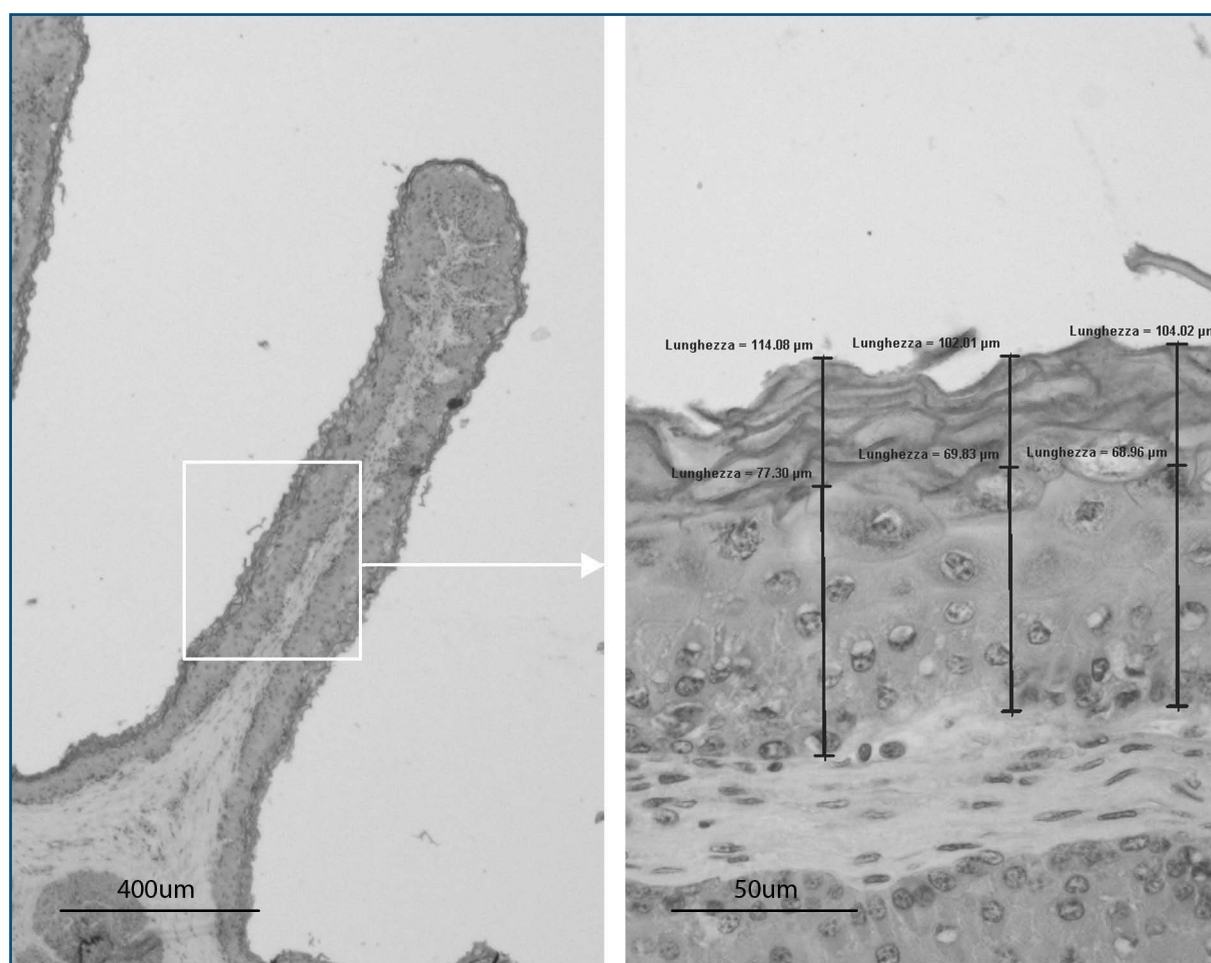


Figure 1. Measuring the degree of keratinisation. The left image shows the selected field where measurement data were collected. The same field is enlarged in the right image. The heights of the total epithelium and nucleated layers are reported.

Japan) and photographed with a digital camera (Dxm 1200, Nikon Corporation). The images were used to measure the keratin layer. The height of the epithelial and nucleated layers was measured, and the height of the keratin layer was calculated as the difference. The values were then converted to percentages of the total height of the epithelial layer. Eight fields of 0.35x0.27 mm were selected for each animal, and five measurements were recorded in each field (Figure 1). The images were processed using image analysis software (Lucia Measurement, Laboratory Imaging Ltd, Praga, Czech Republic).

Body state

The animals were weighed at day 1, 10, and 20 with an electronic balance (Kruuse PS250) and evaluated for BCS. The BCS is a comprehensive assessment of the animal's body status based on relative proportions of muscle and fat, and is considered a useful management tool in determining the welfare of domestic animals (3, 11). In sheep, the assessment is based on the following steps: palpation of body structures, which are represented by the spinous processes of the thoracic vertebrae and the transverse processes of the lumbar vertebrae; evaluation of the muscle/adipose mass in the dorso-medial area; and

evaluation of the general status of the animal. Each animal is graded on a numerical scale (0-5) based on comparison with animals and body structures as described and pictured on a chart. The BCS is the mean of all assigned scores for the four parameters. The animals were scored by five experienced operators, scores were assigned for each parameter by different operators.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the difference between the control and experimental groups. The inter-group data were assumed to be samples from normal populations with the same variances. To test these assumptions, Shapiro-Wilk test of normality and Levene's test of homogeneity were used. When these conditions were not satisfied, a nonparametric Mann-Witney test was applied (20). The analyses were performed using SPSS PC 8.0 software.

Results

During the experiment, the animals in the control and experimental groups were healthy and did not

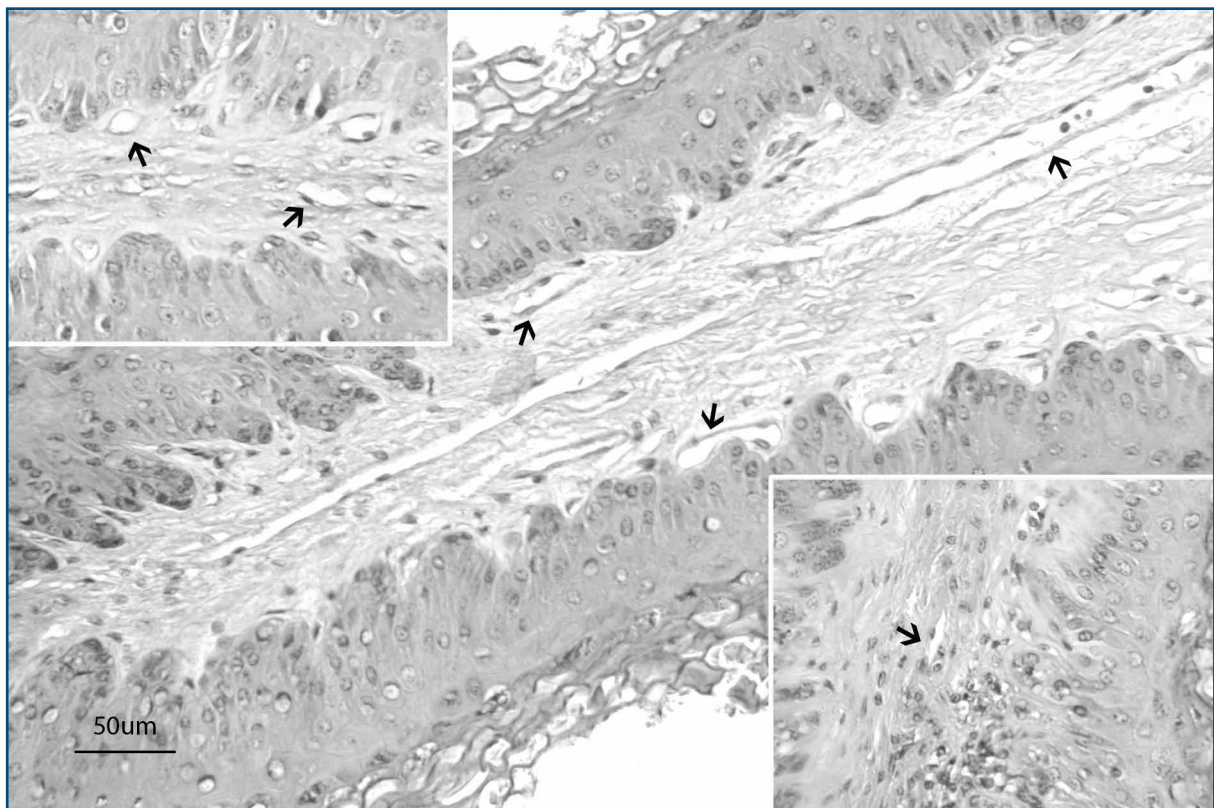


Figure 2. Rumen atrium papilla of sheep grazing on *Brachypodium rupestre*. *Haematoxylin and eosin*. At the beginning of the experiment, numerous large blood vessels (↑) were present in the subepithelia and inner zone of the tunica propria submucosa of the rumen papilla. On experimental day 10 (left inset), the number and calibre of blood vessels (↑) decreased. At the end of the experimental period (right inset), only a few small blood vessels (↑) were present in the inner connective tissue.

show any macroscopic damage to the lip, tongue or buccal mucosae due to feeding. Light microscopy did not reveal any microlesions in the mucosa of the rumen atrium and ventral sac in sheep grazing on *Brachypodium rupestre*.

Histological observation of the rumen mucosa showed a considerable increase in the degree of keratinisation of the rumen epithelium and a decrease in the number and calibre of rumen vessels in the experimental group, as shown in Figure 2. In the control group, the degree of keratinisation was slightly enhanced, and no appreciable differences in the number and calibre of vessels were observed.

The keratin levels from various sites in the rumen measured at three different time points during the experiment are summarised in Table I and shown in Figures 3 and 4. In the rumen atrium, the percentage of keratinisation increased from 17.2% to 31.7%, while in the rumen ventral sac it increased from 20.0% to 37.3%. In the control group, the percentage of keratinisation increased from 17.0% to 19.5% in the rumen atrium and from 20.2% to 22.1% in the ventral sac.

At the beginning of the experiment, there were no significant differences in the percentage of keratinisation between the control and

experimental groups in the rumen atrium and the ventral sac. On days 10 and 20, the percentage of keratinisation showed significant differences in the rumen atrium ($P < 0.001$) and the ventral sac ($P < 0.001$).

The BW trends in the control and experimental groups are reported in Table I and shown in Figure 5. A major decrease in BW occurred during the second phase of grazing on the plot densely covered with *Brachypodium rupestre*. The animals had a total mean BW loss of 1.79 kg. In the control group, BW increased by 0.62 kg, although a slight decrease occurred during the second phase of grazing. Analysis of variance did not show significant differences between the control and experimental groups at any of the experimental time points (Table I).

The BCS trends in the control and experimental groups are reported in Table I and shown in Figure 6. The sheep grazing on *Brachypodium rupestre* showed a decrease in BCS (-0.62) on day 10. The BCS decreased to -1.19 after 20 days. In the control group, BCS decreased slightly (-0.13) on day 20. At the beginning of the trial, there were no significant differences in mean BCS between the control and experimental groups. On day 10, there was a significant difference in mean BCS ($P = 0.003$), which increased after 20 days ($P < 0.001$).

Table I. Mean values of keratinisation of the rumen epithelium (percentage), body weight (BW) (kg) and body condition score (BCS) in sheep that grazed on *Brachypodium rupestre* (Exper) and semi-mesophilic pastures (Control).

Days	Keratin (%)						BW (kg)			BCS		
	Atrium			Ventral sac			Control	Exper	P	Control	Exper	P
	Control	Exper	P	Control	Exper	P						
1	17.0	17.2	0.4030	20.2	20.0	0.5790	50.63	50.83	0.924	2.72	2.75	0.8060
10	19.2	30.9	0.0001	21.7	32.9	0.0001	51.65	51.66	0.994	2.67	2.05	0.0030
20	19.5	31.7	0.0001	22.1	37.3	0.0001	51.25	49.04	0.230	2.59	1.65	0.0001

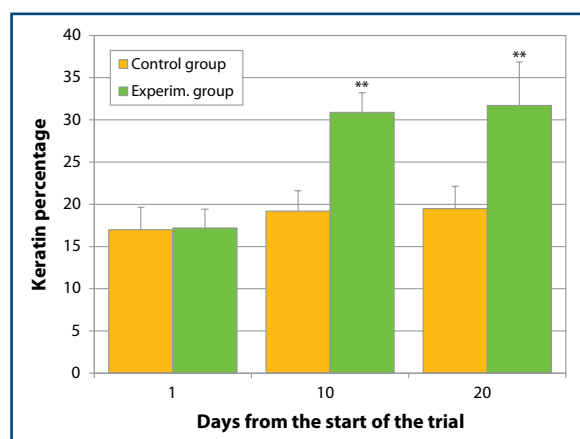


Figure 3. Mean percentage of keratin (\pm SD) in the rumen atrium epithelium in the control and experimental groups on days 1, 10, and 20 (** $P < 0.001$).

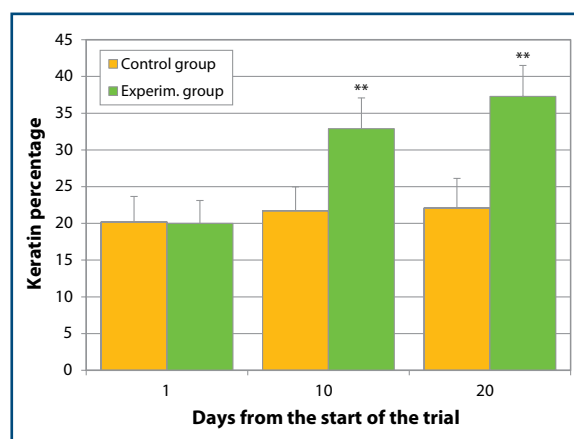


Figure 4. Mean percentage of keratin (\pm SD) in the epithelium of the rumen ventral sac in the control and experimental groups on days 1, 10, and 20 (** $P < 0.001$).

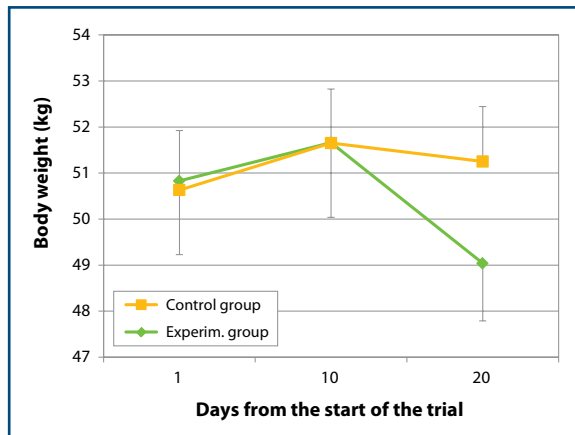


Figure 5. Trends in mean body weight (\pm SD) in the control and experimental groups on days 1, 10, and 20.

Discussion

There were differences observed in the keratinisation of the rumen mucosa and BCS between the control group and the experimental group.

Changes in BW were not significant between the two groups. Although the mean decrease in BW of 1.79 kg was not statistically significant, it should be taken into account as it represents an economic loss for the farmers. The sheep in the control group showed a slight decrease in mean BW at the end of the trial, which was likely due to the lower nutritional value of the plants selected by ewes during the first 10 days of grazing (21) and modifications in the chemical composition of plants due to an increase in cellulose and lignin contents (10).

The greatest increase in keratinisation was observed in the ventral sac of the rumen. Tough and fibrous foods remain longer in the ventral sac, which could explain the differences in keratinisation because direct contact between food and the forestomach wall can induce mechanical stress. A high degree of keratinisation in the epithelial lining was observed after only 10 days of grazing in the plot densely covered with *Brachypodium rupestre* in the rumen atrium (30.9%) and ventral sac (32.9%).

Grazing on *Brachypodium rupestre* in a densely covered plot affected the body condition of the animals within 10 days of exposure. Changes in BCS are considered a more sensitive indicator of the nutritional status of the animals than changes in BW. Body weight takes into account the total weight of the gastrointestinal contents (3). Changes in BW can lead to an over- or underestimation of the welfare of the animal because a relative scarcity of nutrients can be linked to an absolute scarcity of food, and consumption of inadequate food sources can result in a loss or a gain in BW.

Evaluation of BCS is a simple, inexpensive method

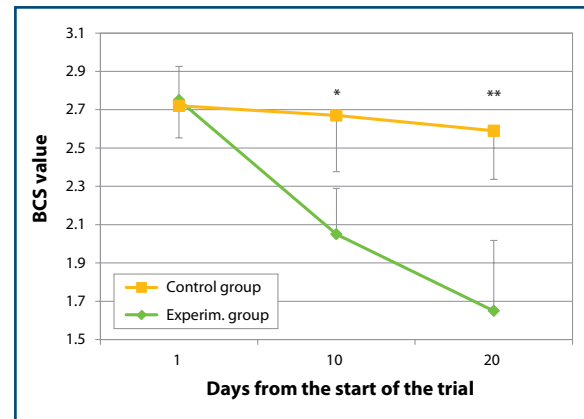


Figure 6. Trends in mean body condition score (BCS) (\pm SD) during the experimental period on days 1, 10, and 20 (* $P=0.003$, ** $P<0.001$) with respect to the control group.

that allows the available reserves to be assessed and indicates the direction (and, to some extent, the rate) of the primary metabolic processes of anabolism and catabolism. The reliability of BCS was confirmed by several endocrine and metabolic blood indicators (3).

A previous study stated that morphometric variations in the mucosa of the ventral sac of the rumen are related to vegetative cycles in the pasture (6). Similarly, changes in BCS observed during vegetative cycles in the pasture were related to mucosal morphometric variations. The degree of epithelial keratinisation in the ventral sac and atrium of the rumen showed the highest mean values when mean BCS was low and the pasture was in a dry phase of the vegetative cycle in 2007, which was a drought year. The lowest degree of keratinisation corresponded with the period when the pasture was blooming in the spring of 2008, which was a rainy year, and mean BCS was higher (6). Body condition score declines in the presence of low quality- and/or low quantity- foraging resources, which directly alters the extent of the absorptive surface of the rumen and the absorption of volatile fatty acids (14, 18). The absorption capacity, which is facilitated by reduced epithelial cell layers (13), is lowered due to increased mucosal keratinisation.

The probable reduction in absorption function observed in the sheep in this study is supported by a decrease in the number of vessels in the rumen tunica propria submucosa during the experimental period, which has been previously verified in animals that were nourished with highly fibrous foods with low nutritional values (13, 14, 15). The control group showed little variation in the degree of keratinisation in the rumen mucosa, which did not alter the absorptive capacity of the rumen or reduce BCS. In contrast, the experimental group showed a considerable increase in the degree of keratinisation in the rumen epithelium due to consumption of a

tough, fibrous food source with a low nutritional value. In the ventral sac of the rumen, the high degree of keratinisation altered the absorption capacity and worsened the body condition during the first and second stage in the experimental plot. The rapid decline in mean BCS in the experimental group was likely due to changes in the structure of the rumen mucosa combined with the low nutritional quality of available food in the pasture, which preceded the weight loss.

Conclusions

The results of the present experiment show that the rumen responded rapidly to mechanical abuse as a result of the highly fibrous *Brachypodium rupestre* leaves. Besides, the increase in keratinisation preceded the decline of other parameters, such as BW and BCS, which were appreciable when the welfare of the animal had already been affected by consuming foods with low nutritional values. Therefore, trends in the keratinisation of the rumen epithelium could be indicative of body state detriment.

Due to the rapid increase in keratinisation and the negative effects on BCS within 10 days of grazing on a pasture densely covered with *Brachypodium rupestre*, animals should not remain in these pasture environments for more than 10-12 days. The assessment of BCS could represent a useful means of controlling the welfare status of sheep grazing in pastures with foraging materials that have low nutritional values to prevent consistent loss of BW.

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