Influence of Feed Particle Size on Magnesium Absorption in Cattle

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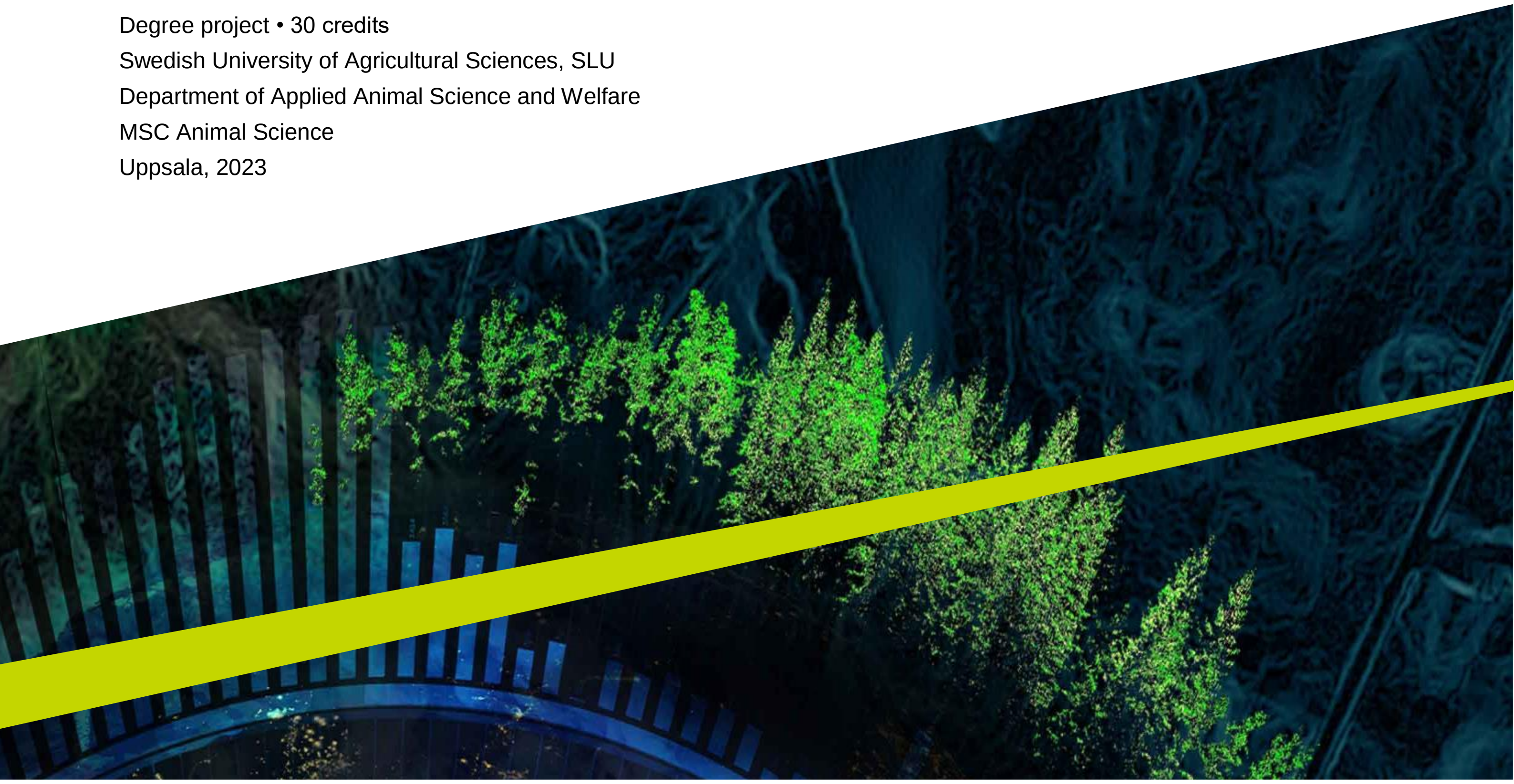

Influence of Feed Particle Size on Magnesium Absorption in Cattle

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1. Abstract

Rumen passage rate depends upon the feed's particle size, specific gravity, and chemical composition. Since rumen epithelium is the leading site of Mg absorption, changes in the rumen passage rate may influence magnesium (Mg) absorption. The study investigated the impact of changes in the forage physical form and maturity on Mg absorption by altering the feed's physical form and NDF contents and monitoring how these two factors could be manipulated to reduce the risk of Mg deficiency in cattle. Eight (8) Swedish Red cattle, four fistulated and four non-fistulated, were enrolled in the study and given the experimental diets for the study period of 21 days. The rumen passage rate was measured using the fibre fraction of the roughages mordanted with 2 grams of Cr through serial faecal sampling for 132 hours after feeding the Cr-marker. The other parameters recorded were daily Mg intake, Mg digestibility, Mg proportion in urine and faeces, dry matter digestibility, Mg excreted in urine, faeces and milk and total mean retention time (TMRT). The statistical analysis using SAS statistical software with the stage of cut (early vs late) and nature of processing (chopped vs extruded) as independent variables showed that the stage of cut influenced the dry matter digestibility, Mg intake and digestibility. Processing, i.e., extrusion, decreased the particle size and lowered the dry matter digestibility compared to the control group. Most dietary Mg is excreted through the faeces, and a smaller proportion is excreted in the urine. The processing method did not influence the proportion of Mg in urine and faeces. Stage of cut had a statistically significant relationship with the Mg proportion in faeces. The smaller fraction of Mg excreted in the milk was independent of the stage of cut and the nature of the processing. The total mean retention time (TMRT), the primary measure of rumen passage rate, depends upon the density and size of the particles but was not affected by the independent variables used in the study. The stage of cut was thus a more important regulator of Mg metabolism than the processing.

2. Introduction

Livestock production is a vital part of the human food chain and a livelihood source for livestock farmers (Castro-Montoya & Dickhoefer, 2020). The ruminants convert roughage that is not digestible for humans into high-quality products. Besides the energy and proteins, ruminants also need minerals, e.g., calcium, magnesium, potassium, iron and phosphorus, for optimum performance (Ensley, 2020). Magnesium is vital in metabolism as it works as a cofactor for hundreds of enzymes in the body. It influences farm animals' production and fertility, improves feed digestibility, and decreases the service period, the time interval between calving and successful conception (Pinotti et al., 2021). The animal body balances its dietary uptake and excretion to ensure optimum levels in different body tissues like muscles and blood. Low dietary magnesium levels cause various issues, e.g., problems with muscle functions, growth, and bone health (Pinotti et al., 2021).

Dairy farmers are facing various issues regarding the metabolic functions of the cow, e.g., milk fever, ketosis, and hypomagnesaemia. Hypomagnesaemia, or grass tetany, is an important issue, particularly in high-yielding dairy animals with high metabolic demand for magnesium and fed mainly herbage-based diets (Martens & Stumpff, 2019). It is particularly experienced in cattle grazing on pastures with high potassium content (Jittakhot et al., 2004), as such a diet reduces magnesium absorption (Xie et al., 2021). A common approach to prevent grass tetany is to increase the magnesium contents in the feed by supplementing concentrate fed to dairy animals with MgO to counterbalance the inhibitory effects of potassium on the absorption of magnesium (Jittakhot et al., 2014). However, there are many other factors to be considered. The available data also suggests that magnesium absorption is influenced by fibre intake and the rumen passage rate (Mann et al., 2019). The rumen passage is the clearance rate of ingested feedstuff from the ruminoreticular region after microbial degradation. The contents are ultimately passed into the omasum. The rumen passage rate depends upon the feed intake and the chemical composition, specific gravity, and particle size of the feed (Godoi et al., 2021). Fibre intake is considered while formulating the diets of dairy animals as it influences the movement and absorption of nutrients, including magnesium, in dairy cattle (Coudray et al., 2003). As the rumen epithelium is the main site of magnesium absorption, increasing the rumen passage rate decreases the contact time between rumen epithelium and rumen contents and changes the absorption of magnesium from the rumen (Martens et al., 2018; Leonhard-Marek et al., 2010)

The rumen passage kinetics, i.e., the passage rate and the rumen volume, may influence the absorption of magnesium from the rumen. The important rumen passage kinetic parameters are the passage rate in the liquid phase (KP_L), liquid phase volume (Vol_L) and passage rate in the solid phase (Kp_S) (Lopes et al., 2015; Kuoppala et al., 2010; Schonewille et al., 2002; Krämer et al., 2013). These parameters influence the rumen passage rate differently and are influenced by the nutritional characteristics of the feed. The fibre content and particle size of the feed influences the rumen passage rate, and it can be changed to alter the passage rate and, ultimately, the Mg absorption in the rumen (Sniffen & Robinson, 1987).

3. Aims and objectives of the study

Although vast literature is available about the influence of rumen kinetics on the absorption and excretion of nutrients, more research is needed to see the effects of altering the rumen passage rate through changes in the NDF contents and physical forms of the feed on the magnesium absorption. This study was planned to see how these two factors influence the absorption of magnesium in the rumen and how these factors can be manipulated to reduce the risk of Mg deficiency in dairy cattle.

4. Rumen passage rate and nutrient digestibility and availability

The ruminants are unique due to the nature of their digestive system in which microbial degradation in the fore-stomachs plays a vital role. The ruminants can utilise the fibrous plant materials because they stay in the rumen for a very long time, giving the rumen microflora time to degrade it. The feed residues are cleared from the rumen at a variable rate known as the rumen passage rate (Kp) to allow more intake (Krizsan et al., 2010). Several approaches are used to estimate rumen passage rate, e.g., the flux/ compartmental pool method described by Ellis et al. (1994). The knowledge of Kp is very important to predict the ruminants' feed utilisation and feed efficiency. Two types of factors influence the rumen passage rate: intrinsic and extrinsic. Common extrinsic factors are the feed particles' specific gravity, the particle size reduction rate, and the feed particle size itself (Huhtanen et al., 2006). The extrinsic and intrinsic factors are independent, but both can influence feed digestibility. The other factors, e.g., the forage species/ varieties, stage of maturity, primary growth or regrowth, and the leaf-to-stem proportion, have been suggested as determinants of the intrinsic characteristics that can influence the Kp of the rumen particulate matter (Kuoppala et al., 2009: Kuoppala et al., 2010). Various prediction equations have been established to determine Kp, which are based on a very large data set using either Cr-mordanted fibre or rare earth as markers of Kp (Seo et al., 2006). Irrespective of the factors influencing the rumen passage rate, dietary manipulations, e.g., changes in the particle size and fibre content, are used to influence it and thus influence the digestibility and availability of the nutrients.

5. Feed particle size and the rumen passage rate

The ruminoreticulum's clearance rate depends upon the feed's physical characteristics and the animal's physiological demands. The smaller forage particles have a higher passage rate than the larger particles, resulting in lower rumen pH and shorter rumination time (Zebeli et al., 2007). Besides the particle size, the particle density also influenced the passage rate, and Dufreneix et al. (2019) noted that the particle size did not influence the total mean retention time (TMRT) when the density of the particles was between 1.1-1.3. However, outside this range, increasing the particle diameter significantly increased the TMRT and thus lowered the passage rate. To allow a steady flow of content, the ruminants use various mechanisms to reduce particle size, e.g., mastication and digestion. Before the passage of materials from the rumen, the particle size must be reduced to less than 1200 µm. After mastication and digestion, the particle sizes of forages become smaller but still have considerable variations in size and can be between 200-1200 µm (Martz & Belyea, 1986). The feed's particle size also influences the rumen's particle size. Rumination and mastication reduce the variations in particle sizes. Although the ruminants spend considerable energy to move the materials, the propulsion of materials is also influenced by many factors, e.g., pH of the rumen, osmotic pressure in the rumen, cell wall percentage, and the density of the feed particles. Besides the rumen passage rate, the feed particle size also influences the chewing time. For example, Ramirez et al. (2016) noted that feeding smaller particle sizes (just 0.3% particles greater than 19.0 mm) reduced the chewing time from 720 minutes to 570 minutes daily. Using more extended particle size (5.4% particles greater than 19.0 mm) caused a decrease in the rumen passage rate from the original 3.38%/h to 2.89 ± 0.42%/h.

Similarly, a higher percentage of the cell wall (particularly the lignified walls) results in low digestibility and slower passage. The pH and osmotic pressure of ruminal contents influence the movements of the intestinal muscles and digestive efficiency, as optimal pH and osmotic pressure support peristaltic movements in the rumen by supporting microbial fermentation and maintaining the fluidity of the ruminal contents. Hence, both factors ultimately influence the rumen passage rate (Jung, 2012). Besides the particle size, the forage family also influences the rumen passage rate and the dry matter intake (DMI). It was explored by Kammes and Allen (2012), who noted that the passage rate and the reduction in particle size through mastication were higher for legumes than cool season grasses, resulting in low fibre digestibility and lower rumen fill when feeding legumes. It was found that a diet consisting of orchardgrass silage (OG), a cool season grass, resulted in an increased rumen pool of larger NDF particles as compared with the alfalfa silage (a legume). So, although the particle size influenced the rumen passage rate, it was not the only constraint. Different forage families also have different passage rates. In simple words, the characteristics of the feed have an enormous influence on the rumen passage rate.

6. Role of magnesium in ruminant nutrition

Magnesium is essential for various biological functions. It plays a role in more than 300 enzymes in the body. Hypomagnesaemia develops if the magnesium outflow in the faeces, milk, and urine exceeds its inflow through the diet or if there is an acute redistribution of Mg in the body (Doncel et al., 2021). Appropriate levels of Mg in the diet are necessary to ensure health maintenance and adequate growth. The farm animals' performance improvements have increased the metabolic demands of Mg. It is recommended that the Mg supplementation in the diet must be adequate to ensure the optimum health of the animals and the quality of the products (Schonewille, 2013). Although there are animal-to-animal variations, the average Mg content in the bodies of most farm animals is 0.4 g/ kg body weight (Maguire & Cowan, 2002).

Mg is essential in farm animals' bone development and cellular metabolism (EFSA, 2015). Various salts of Mg, including sulphates, carbonates, and oxides, are used for dietary supplementation, and all are good sources of Mg for farm animals. Among these sources, MgO is the most commonly used mineral available as a source of Mg (EFSA, 2015). The MgO allows sufficient absorption of the Mg ions. However, not all MgO sources are equivalent to providing the necessary Mg^{2+} ions vital for living cells. Different MgO products differ in various characteristics, e.g., bioavailability, reactivity, and solubility (Suttle, 2022). The Mg bioavailability of different mineral compounds is also different. For example, the Mg bioavailability of magnesium oxide and magnesium sulphate are 20% and 45%, respectively (Lipinski et al., 2011).

Various factors compromise the absorption of Mg from the rumen epithelium, the main site of absorption. It is widely accepted that the absorption efficiency of Mg determines the supply of Mg to the cells. Consequently, insufficient absorption of Mg is widely known to be the most important cause of Mg deficiency, leading to clinical conditions, e.g., milk fever and grass tetany (Doncel et al., 2021). Common symptoms of grass tetany are staggered walking, muscle twitching, and nervousness. In the later stages, convulsions and muscle spasms may cause the animal to lie down on its side, and death can occur in untreated cases. Sometimes, the death is so quick after the onset of clinical signs that the animal is found dead without noticeable signs. Older animals are more susceptible to more lethal episodes because, unlike the younger animals, they cannot mobilise Mg from the bones quickly. However, animals of all ages are susceptible (Elliott, 2009). Although quick treatment by a veterinarian will allow the animal to recover rapidly, the best option to avoid it is to feed a mineral supplement that provide a high concentration of Mg. The high Mg minerals have at least 12-14% Mg in them and are more effective for the job. All of the Mg taken in through the feed does not reach the blood; the majority is excreted through the faeces. As Mg is absorbed in the rumen, and the absorption is not well regulated, an increased passage rate in the rumen will likely reduce the Mg absorption and increases its excretion in faeces. Various studies have noted a relationship between Mg intake and its excretion in the faeces (Schonewille, 2013; Coudary et al., 2005).

7. Magnesium absorption in the rumen

The primary site of absorption of Mg^{2+} ions is the rumen. In the rumen, it is absorbed using both the potential difference-independent (PD-independent pathway) and potential difference-dependent (PD-dependent pathway) pathways. These pathways involve the movement of electroneutral transporters and ion channels, respectively (Fontenot et al., 1989). The net absorption of magnesium at various sites in the digestive system results from two unidirectional flows; one from the blood to the lumen and the other in the opposite direction. The electrogenic mechanism works at lower concentrations of Mg in the rumen and drives the Mg ions passively into the ruminal wall cells. The electroneutral mechanism is responsible for the majority of Mg uptake in sheep. It is linked to the transport of carbon dioxide (CO_2) and short-chain fatty acids (SCFAs) that indirectly stimulate the absorption of Mg by activating the H^+-ATPase system. It is also well established that the extrusion of Mg from the ruminal cells into the bloodstream is carried through an active process that uses ATP energy and is linked closely to the Na,K-ATPase. Opposite to this flow, the back secretion of Mg into the rumen takes place along an electrochemical gradient through paracellular space resulting in a steady but small loss of Mg (Fontenot et al., 1989).

8. Various factors influencing the absorption and excretion of magnesium

Like other minerals, the absorption and availability of magnesium are influenced by many factors. The potassium content of the feed is known to be a risk factor for hypomagnesaemia, as the potassium reduces the digestibility and absorption of the Mg (Martens & Schweigel, 2000). The potassium blocks non-specific transporters involved in magnesium absorption and alters the potential difference, influencing the Mg absorption (Martens & Schweigel, 2000). To offset the problem caused by dietary potassium, different sources of magnesium are added to the diet to reduce the risk of potassium-induced hypomagnesaemia. Similarly, the rumen passage rate also influences magnesium absorption. The excretion of Mg in the faeces and the urine is an important indicator of digestion as Mg in the faeces is undigested, and the Mg in the urine has been digested. Weiss (2004) collected data from 8 different studies on lactating cattle. The magnesium digestibility in all these studies was determined by estimating the Mg contents of the faeces. It was noted that the apparent digestibility of magnesium decreased linearly by 0.075 with each percentage increase of K in the diet. Another nutritional study has shown that increasing the K concentration in the diet (6, 24, 48 g/kg DMI/ day) resulted in a three-fold decrease in the apparent Mg availability (Greene et al., 1983). It is also important to note that the malabsorption of Mg at the ruminal epithelium is not compensated at the intestines. Any disturbance of the Mg uptake is balanced by changing the urinary excretion of Mg, so the Mg in urine is a good indicator of its digestibility (Martens and Schweigel, 2000). Due to the adjustment through urinary excretion, Mg toxicity is not a known clinical problem. However, excessive Mg in the feed reduces its palatability and can cause diarrhoea (Schonewille, 2013).

9. Materials and Methods

The initial experiment and sampling were conducted at the Swedish Livestock Research Centre, Uppsala, the Swedish University of Agricultural Sciences (SLU) research facility, from January 2020 to April 2020 as described by Managos (2020). The experiment was originally conducted to see the effects of the extrusion of grass silage on the ingestive behaviour of the cattle, milk production and feed intake. The data from the same experiment were used in this study to determine the rumen passage rates in the experimental animals and see how the treatment influences the magnesium absorption from the rumen epithelium. The experiment and all the procedures related to the handling of the animals were conducted after the approval of the Local Ethics Committee of Uppsala, Sweden. The identity number of the approval is 5.8.18-12171/2018.

10. Animals and housing

Eight multiparous lactating cattle of the Swedish Red breed were used in the study. Four cows were fistulated (days in milk-DIM: 145 ± 39), and the remaining four were intact (DIM: 70 ± 11). The mean lactation numbers of the fistulated and intact cows were 2.58 ± 0.96 and 2.5 ± 0.58. The cannulation was performed not later than 5 months before the start of the experiment. One cow in the fistulated group had experienced mastitis in the current lactation, and one of its udder quarters was non-functional. The animals were kept in the individual tie stalls (length: 1.8m, width: 1.6m), and additional space was provided by adding a platform of 0.6m width at the end of the stalls. The stall temperature was maintained at 8-15^{o}C, and an empty stall was kept between each animal. Each animal was provided with automatic water containers with water meters (model P-50, Schlumberger Ltd, France).

The animals were fed concentrates and forage in separate troughs while the blocks containing sodium chloride were available for licking in each stall. The animals were relocated to these tie stalls 10 days before the initiation of the experiment to allow them to adjust properly to the new housing conditions. Milking was carried out twice daily at 8:00 and 18:30.

11. Study design

A Latin square design was used with 4 treatments and 4 periods. Each of these experimental periods was of 21 days. The first 14 days were provided to the cattle as an adaptation period to each new feed, and the sampling was done in the last 7 days. The *ad libitum* silage was offered to the cows at two different maturity stages (late harvest and early harvest) and in two forms (chopped and extruded) in a 2x2 arrangement involving 4 different diets. The treatments were allocated randomly at the experiment's start and changed periodically according to the design. Besides silage, the animals were also fed concentrates, including a compound feed, soybean meal and a mineral mix. The composition of the compound feed is given in Table 1. The intact animals received 10 kg of concentrate daily (2 kg soybean meal and 8 kg compound feed) because they had higher milk production earlier in the lactation. The fistulated animals received 8 kg of concentrate daily (2 kg soybean meal and 6 kg compound feed). The silage was given thrice a day (at 7:00, 12:00 and 20:00), and the concentrate was given four times each day (7:00, 12:00, 16:00 and 20:00). If required, the animals were offered additional silage to allow ad libitum feeding and the leftovers were collected before the first feeding in the morning.

Table 1: Composition of the compound feed (per Kg).

Ingredients	Percentage
Rapeseed meal	24.1
Barley	36.3
Wheat bran	15
Wheat	5
Oats	10
Sugar beet pulp	1.9
Molasses	2
Vitamins and minerals	3.8
Fat	1.9

12. Silage preparation

The silage was prepared from the early and late stages of harvest of the seasonal grass. The purpose of the two harvest dates was to investigate if there was an interaction between the effects of maturity and extrusion on cow performance. Wilting was done to achieve the desired dry matter content of 40-50%, and it was then preserved in the form of large bales 120 cm by 100 cm in size and was wrapped in 8 plastic layers. The storage was done in a concrete area and kept away from the reach of wild birds. Before harvesting for botanical analysis, and the composition was 70% Timothy, 26% Tall Fescue, 3% Red Clover and 1% Weeds. The stage of maturity of Timothy was determined by using the method described by Pomerleau-Lacasse et al. (2017), who observed 78 % of Timothy at the reproductive stage (first cut, R0 42%, R1 19%, R2-R3 17%) and 22% of Timothy at elongation stages (late cut, E4-E5). The results from the nutritional analysis are represented in Table 2. The results from the nutritional analysis are described in Table 2.

Table 2: Composition of silage and concentrate feed used in the feeding trial.

	Silage				Concentrate	
	Early harvest		Late harvest		Compound feed	Soybean meal
	Chopped	Extruded	Chopped	Extruded		
Dry Matter (DM), %	45.8	47.7	50.5	52.4	89.4	89.5
Ash, % of DM	8.0	8.0	7.1	6.9	7.2	6.7
Crude Protein (CP), % of DM	12.5	12.4	10.6	10.5	19.4	51.6
Soluble CP, % of CP	46.3	43.1	40.7	36.5		
NDF, % of DM	54.8	54.9	55.1	55.2	24.9	16.1
IVOMD[2], %	79.9	80.1	71.3	71.2		
ME[3], MJ/kg DM	10.0	10.0	8.8	9.0		
pH	4.9	4.9	5.3	5.3		
NH3-N, g/kg N	31.7	29.0	22.1	19.7		
Lactic Acid, g/kg DM	19.8	18.5	8.5	7.9		
Acetic Acid, g/kg DM	5.6	5.1	2.8	2.6		
Propionic Acid, g/kg DM	2.8	2.5	2.4	2.3		
Butyric Acid, g/kg DM	0.5	0.4	0.2	0.2		
Ethanol, g/kg DM	3.3	4.1	4.2	5.6		
Ca, g/kg DM	3.5	3.5	3.2	3.2	9.7	3.7
P, g/kg DM	2.4	2.4	1.9	1.9	6.7	6.7
Mg, g/kg DM	1.4	1.4	1.3	1.3	4.3	3.4
K, g/kg DM	28.2	28.3	24.5	24.5	9.1	23.5

[1]Complete Norm 180, Lantmännen; [2]In Vitro organic matter digestiblity, determined after 96 hours of incubation in rumen fluid (Lindgren, 1979); [3]Metabolisable energy, calculated as IVOMD*0.16-1.91 (Spörndly, 2003).

13. The extrusion process

The processing of silage was performed two times each week during the experiment. Before the processing, the bales were opened and closely monitored for signs of mal fermentation. Areas affected by mal fermentation in each bale were discarded, and the whole bale was dumped in case these areas were large. Bales were first processed in a mixer wagon to reduce the particle size, and silage was then processed in the extruder. The extrusion was done in the bio-extruder (MSZ-B15e, LEHMANN GmbH). The speed of the rotation was fixed at 54 RPM, and the ending opening of the extruder was adjusted to 50%. An infrared thermometer gun (STANLEY STHT77365) was used for the measurement of temperature during the processing of the late harvest (LE-3 recordings) and the early harvest (EE) (4 recordings) silage. The silage temperature was recorded as it was exiting the extruder, and the mean values were 52°C±2 SD for EE and 57°C±2 SD-LE. The mean values at the midpoint of the extruders' barrel were 43°C ±4 SD for EE and 54°C ±3 SD-LE, and the mean values of temperature at the exit point of the barrel were 60°C ±3 SD for EE and 63°C ±2 SD-LE.

14. Sampling and measurements

The weights of the feed being offered, and the refusals (concentrate and forage) were recorded daily (on a total fresh matter basis) throughout the experiment. Samples were collected from the offered and refused silage during sampling weeks throughout the experiment. Similarly, the samples were taken from the leftover concentrates and pooled within animals and periods. A screen (1 mm) was used to mill these samples, and they were then analyzed for acid-insoluble ash, ash, Kjedahl-N, and DM. Spot samples of the faeces and urine were collected twice daily (9:00 and 17:00) from day 15-19 of the experiment.

15. Handling and analysis of samples

The urine samples were diluted (1 ml urine diluted in 9 ml of water). The plastic bags were used to collect the faecal samples (450 ml). The faeces and urine samples were stored at -20^{o}C until further processing. The compound concentrate, soybean and mineral samples were pooled towards the end of the experiment. Two further sub-samples were created from them, 250g for compound feed and soybean meal and 100g for the minerals within the period and feed type. One of these sub-samples was stored at -20^{o}C for future analysis, while the other was used to analyse the mineral composition, NDF, Kjedahl-N, acid insoluble ash, ash and DM.

The silage samples were collected and stored (at -20^{o}C) daily. A sub-sample of 0.5kg was frozen at -20^{o}C for any analysis in the future, and the rest were pooled for treatment. The pooled samples were milled using a 13 mm screen on the meat mincer and were divided into three further samples. The larger of these samples (400g) was used to analyse minerals, NDF, DM, ash, acid-insoluble ash, Kjedahl-N and IVOMD and another smaller 20g sub-sample was stored at 4^{o}C after mixing with the equivalent amount of water. The hydraulic press was used to extract the silage liquid, which was used to analyse alcohols, lactic acid, pH, VFA, NH_4 and Kjedahl-N.

The faecal samples were gradually thawed and then pooled gently. A 0.5 Kg sub-sample was frozen at -20^{o}C for any further analysis in the future. The water was mixed with the remaining pooled sample (at the rate of 10% of the total faeces weight) and mixed vigorously with an electric drilling machine. Another sub-sample of 1 Kg was also collected and frozen at -20^{o}C, and 120 g was placed into two large petri dishes for freeze-drying and the analysis of the ash and acid-insoluble ash.

The passage rate in the solid phase was measured using the Cr- mordanted fibre fraction of the roughages prepared using the method described by Warner et al. (2013). On day 1, a pulse-dose with NDF bound to 2 grams of Cr was injected into the rumino-reticulum of the dairy cow through the cannula. The quantity of the marker being administered was always kept within the non-toxic limits (NRC, 2001). After the marker administration, a series of faecal samples were collected at 20 different time points during the whole 132-h period, with the target hours being 0, 12, 16, 20, 24, 28, 32, 36, 44, 52, 60, 68, 76, 84, 92, 100, 108, 124, and 132. If no defecation occurred at the sampling time, the faecal sample was collected directly from the rectum. As the actual sampling time showed a variation of ±8 minutes from the target time, the exact time for the sampling was recorded, and corrections were made before the statistical analysis. All of the faecal samples thus collected were placed in the Petri dishes, and the weight of each sample was recorded than the samples were stored at -20^{o}C. All of these collected samples were freeze-dried, ground and dispatched to the commercial lab (ALS Global, Luleå) to analyze Cr concentration using the plasma emission spectroscopy method. The Cr concentration in these samples was used to determine the rumen passage rate in the solid phase.

The urine samples pooled by the cow and period were analysed in the university lab to analyse Mg contents using a colorimetric method based on xylidyl blue (Randox, Antrim, UK).

16. Further Calculations

The information provided by the weight of feed offered, feed refusals and DM content of the feed was used to calculate the regular DM intake of both concentrate and silage. The daily dry matter intake during the experiment was used to calculate the average DMI on days 16 to 19 for each animal. The Cr analysis of the faecal samples was used to calculate the rumen passage rates as done by Eklund (2020) using the prediction equations developed by Cornell Net

Carbohydrate and Protein System (CNCPS) and National Research Council (NRC) (NRC, 2001; Fox et al., 2004).

17. Statistical analysis

All the statistical calculations were performed using SAS statistical software (SAS, 2007). The particle shapes (chopped vs. extruded) and the stage of maturity (early cut vs. late cut) were related to the outcome variables as independent fixed factors. Statistical modelling was done through Proc Mixed in SAS, also including cow ID and period as fixed variables.

18. Results

The results of various variables are summarised in Table 3.

Table 3. Intake, excretion and digestibility of Mg, dry matter digestibility and total mean retention time. Least square means and P-values of comparisons.

	Chopped		Extruded		P-values		
	Early cut	Late cut	Early cut	Late cut	Cut	Process	C*P
Mg intake^1	63.2	60.4	62.9	61.1	0.02	0.83	0.54
Mg digestibility (%)	22.4	18.4	19.7	17.8	0.02	0.20	0.38
Mg urine^2	0.07	0.07	0.08	0.08	0.29	0.17	0.57
Mg faeces^2	0.77	0.82	0.80	0.82	0.02	0.20	0.38
Mg excreted in the milk^2	0.04	0.04	0.04	0.04	0.70	0.05	0.24
Mg in urine^1	4.7	4.7	5.0	5.2	0.72	0.23	0.60
Mg in faeces^1	49	49.2	50.2	50.1	1	0.3	0.8
TMRT^3	48.2	46	51.6	47.3	0.24	0.38	0.69
Dry matter digestibility	0.68	0.63	0.65	0.61	<0.0001	0.0004	0.84

Note: 1: grams/ day; 2: proportion; 3: hours

Mg digestibility was calculated in percentages. The values showed variations among early cut, late cut, chopped and extruded groups. The p-values for cut (early and late cut) were less than 0.05, which means the cut stage had a statistically significant negative effect on Mg digestibility, with lower digestibility for later cut. Neither processing nor cutting*processing interaction had a significant association with the Mg digestibility. Mg intake was calculated in grams per day. The p-values for cut (early and late cut) are less than 0.05, which means the stage of cut had a statistically significant relationship with the Mg intake. Neither of the other factors, i.e. processing nor cutting*processing, were significantly associated with the Mg intake.

Mg proportion in urine means the amount of Mg excreted in urine as a fraction of total Mg consumed. Only a small proportion of Mg was excreted in the urine. None of the three independent variables, i.e. stage of cut (early vs late), nature of processing (chopped vs. extruded), and cut* processing interaction, had statistically significant relationships with the Mg proportion in urine. Mg proportion in faeces means the amount of Mg excreted in the faeces as a fraction of the total Mg consumed. Most of the dietary Mg was excreted in the faeces. The stage of cut had a statistically significant relationship with the Mg proportion in faeces, and the other two factors, i.e., processing and cutting*processing interaction, had a non-significant relationship.

Total Mg excreted in the urine was measured in grams per day. The statistical analysis showed that all of the independent variables, i.e., stage of cut (early vs. late), nature of processing (chopped vs. extruded), and cut*processing interaction, had statistically non-significant relationships with the amount of Mg excreted in the urine.

Total Mg excreted in the faeces was measured in grams per day. The p-values showed that none of the independent variables had statistically significant relationships with the amount of Mg excreted in faeces. Dry matter digestibility was measured in proportion. The values showed variations among the groups. The stage of cut and processing had a statistically significant relationship with dry matter digestibility.

Total mean retention time (TMRT) was calculated in hours. The p-values for all parameters were greater than 0.05, which means that none of the independent variables had a significant relationship with the TMRT. Mg excreted in milk was calculated in proportion. The p-values for all parameters were greater than 0.05. It means none of the independent variables had a significant relationship with the Mg proportion in milk.

19. Discussion

Extrusion increases the surface area of the feed particles, thus allowing an efficient fermentation by the ruminal microflora. However, the extrusion did not increase the digestibility in this study, even if the particle size was markedly reduced. Extrusion decreased the digestibility, and the difference was statistically significant. Grasses cut at the early stages are more digestible due to the lower content of structural carbohydrates (Holden, 1999; Al-Arif et al., 2017). The statistical analysis showed that the stage of cut significantly influenced the dry matter digestibility in this study. This finding conformed with the conclusions of previous studies (Pritchard et al., 1963; Balde et al., 1993), which noted that the digestibility was higher in the early cut forage.

The Mg digestibility means the percentage of dietary Mg that is digested and absorbed by the digestive system. The Mg digestibility is influenced by many factors, including the dietary intake of other minerals, e.g., potassium (Weiss, 2004). The Mg digestibility was higher in the early-cut forage (22%) than in the late-cut forage (18%). The decrease in digestibility in the later cut could be attributed to various factors, e.g., the increased fibre content of the forage, increased mineral binding with other nutrients, poor digestion due to high lignin content, higher resistance to the digestive enzymes and microbial degradation due to thicker cell walls and high lignin contents (Kuoppala et al., 2009). The same findings were reported earlier by Pendulum et al. (1980), who reported that the digestibility coefficient of Mg decreased when the dough stage of tall fescue was fed compared to the earlier vegetative phase. Likewise, it was noted in our study that the extrusion resulted in an insignificant change in digestibility. This finding confirmed the previous findings by Gonthier et al. (2004), who found that the extrusion process did not influence the ruminal digestion of minerals in the flaxseed. The statistical analysis showed that the stage of cut had a statistically significant impact on the Mg digestibility. Similarly, the combination of both independent variables also did not statistically significantly impact the Mg digestibility.

The cut stage had a statistically significant influence on the daily Mg intake. The results conformed to the previous findings by Mirzaei (2012) who noted an increased risk of grass tetany in cattle given mature grasses and lower risk in the cattle fed legumes and early cut grasses. The daily Mg intake was measured in grams. It showed a similar trend to the Mg digestibility. The early cut silage contained the highest amount of Mg. It resulted in the highest daily intake (63g). The intake was lower in the silages prepared from late-cut grasses (61g in the extruded and 60 in the chopped), which might be due to thick and lignified cell walls, making them resistant to digestion and extract nutrients and less appealing to the animals, resulting in low DM intake. Vinyard et al. (2018) noted that the Mg contents in Eragrostis tef changed inconsistently with the stage of maturity. It increases from 0.19% at the boot (BT) stage to 0.20% at the early heading (EH) stage and then decreases to 0.17% at the late heading (LH) stage. It was concluded that the Mg contents of different stages of cut of Eragrostis tef had no effect on the daily Mg intake, and only the total DM intake influenced the daily Mg intake.

The dietary Mg is excreted through urine and faeces. The kidneys filter the Mg, but most of it is re-absorbed as 20-30% is re-absorbed back from proximal tubules and the remaining 60-70% from the ascending region of the loop of Henle (Houillier, 2014). Thus, only a tiny fraction of dietary Mg is excreted in the urine. Holtenius et al. (2008) noted that urinary excretion of Mg increases as the plasma concentration increases due to higher dietary intake and absorption from the gut. The urinary fraction disappeared below a plasma level of 0·61–0·73 mmol/L. Therefore, they suggested that Mg supplementation should start as the plasma Mg level becomes equal to or less than 0.8 mmol/L. We found that various stages of cut did not influence the Mg excretion through urine. The established data predict that the urinary excretion must be lower in the cow fed the late-cut silage because of more mineral binding due to lignification's. The effect of processing was insignificant. More studies involving monitoring other minerals, particularly K and the serum mineral profile, are recommended to explore the causes of high Mg excretion in the urine of animals fed late-cut grasses. The independent parameters, i.e., the cut stage, the nature of processing and both combined, had no significant effect on the Mg in urine. When the total Mg excreted in the urine daily was calculated, it showed no significant differences. However, no previous studies have explored the impact of processing and stage of cut on the Mg excreted in the urine, and more comprehensive studies are needed to understand the influence of these parameters.

Most dietary Mg is excreted in the faeces, and the data showed that more than 75% (77.5% in the early cut and chopped vs. 82.1% in the late cut and chopped) is lost through the faeces. The reason is that Mg retention in the large intestines is less efficient than renal re-absorption. Similar results were reported by Shockey et al. (1984), who noted that 74% of the Mg intake is lost into faeces, and the Mg excretion changes linearly with the dietary Mg intake. The changes were more likely related to the dietary K levels and less to the cut stage. It confirmed our findings that the cut stage has a significant influence on the Mg excreted in the faeces. Shockey and his colleagues (1984) recommended dietary K adjustment to balance the Mg digestibility. On average, each cow excreted 50g of Mg in the faeces, the highest being in the early cut and extruded group.

Besides urine and faeces, a substantial amount of Mg is excreted in the milk of the lactating animals. The Mg concentration in the milk is higher than the plasma levels, and the trait shows high heritability (0.60) in cattle (Van Hulzen et al., 2009). The fraction of Mg intake excreted in milk in our study varied non-significantly between different groups. The previous findings by Cerbulis and Farrell (1976) and Schonewille and Beynen (2005) noted that lactating cows lose around 99-120mg of magnesium in each litre of milk. So, assuming a 30-litre average daily production, the total Mg excreted at 3-4g. Suppose we calculate the recommended daily intake of Mg at 2g/ kg DM as Martens et al. (2018) recommended. In that case, it is roughly 7-8% of total Mg intake and is higher than the proportion observed in our study. The risk of grass tetany is thus substantially higher in lactating cattle, losing a considerable fraction of dietary Mg into the milk.

The total mean retention time (TMRT) measures the rumen passage rate and depends upon the feed particles' density and size. The ruminal bacteria use this time to ferment the ingesta and break down the particles before they move into successive compartments. Although the extrusion is supposed to reduce the size of the feed particles and allow better fermentation, its influence on the TMRT in our study was not statistically significant. It could be attributed to study limitations, e.g., a small sample size. The stage of cut did not significantly influence the TMRT. The results of previous studies were inconsistent. These findings were contradicted by de Silva et al. (2022), who noted that the silage prepared from early cuts of Johnson grass has a higher nutritional value and lower retention time than those prepared at the later stages. The higher nutritional value and lower retention time are attributed to easily digestible carbohydrates in the early-cut grasses. Higher fibre contents and more complex lignified carbohydrates in the late cuts are responsible for higher retention time. As no regular trend was observed, the changes were likely the result of unknown errors and factors related to the processing and handling of grasses and the individual variations among the animals. The small sample size allows random variables to exert more effect and could be minimised using a larger sample size. Therefore, future studies involving a larger sample size are recommended to roll out such factors.

20.Conclusion

The stage of the cut influenced the dry matter digestibility as it decreased with the advanced stages of the cut. However, extrusion did not increase the dry matter digestibility despite increasing the surface area of the particles. The stage of cut also influenced the daily Mg intake and digestibility. However, neither of these parameters was affected by the processing. Most Mg was excreted in faeces and only the Mg proportion in faeces was influenced by the stage of cut. None of the treatments affected the amount of Mg excreted in the milk. Like the Mg in milk, the TMRT was not influenced by cut stage or extrusion.

21. References

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