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Effect of Growth Stage at Harvest and Type of Additives on Nutritional Quality and Fermentative Characteristics of *Hypernia rufa* Silage

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Abstract: The experiment was conducted to determine the effect of harvesting stages and type of additives on nutritional quality and fermentative characteristics of silage of *Hypernia rufa* which is abundantly found tropical grass in Benishangul-Gumuz. Factorial combination of three harvesting stages and four additive types (including control) was used with three replications. Harvesting stages were early vegetative, boot and full bloom stages whereas additives were 4% molasses, 1% urea, 4% molasses plus 1% urea on fresh biomass basis and without additive (control). The grass material was chopped and packed in PVC containers of 30L and stored for 42 days. The dry matter (DM) content of silage linearly increased with increasing level of harvesting stage. Inclusion of molasses plus urea and molasses also increased the DM content of silage. Addition of molasses improved fermentative characteristics and crude protein content whereas inclusion of urea improved crude protein content but negatively affected fermentative characteristics. Neutral detergent fiber and acid detergent fiber increased with increasing level of harvesting stage affecting *in vitro* dry matter digestibility linearly. Type of additives did not affect neutral detergent fiber, acid detergent fiber and in vitro dry matter digestibility. Higher amount of ash was recorded in silage irrespective of harvesting stage and type of additive. In conclusion, this study indicated that appropriate silage can be made from *Hypernia rufa* by harvesting it at early vegetative stage and adding molasses at 4% on fresh biomass basis.

Key words: Acid detergent fiber · Benishangul-Gumuz · Crude protein · *Hypernia rufa* · *in vitro* dry matter digestibility · Neutral detergent fiber

INTRODUCTION

Feed is one of the major constraints limiting livestock productivity in Ethiopia. Nevertheless, adequate feeding is essential for sufficient milk production, high rates of weight gain, fast growth, efficient reproduction and disease resistance. The major feed related problems in Ethiopia that are hindering the livestock productivity include seasonal variation in availability of green feeds, poor nutritional quality of feeds, unavailability and unaffordability of concentrate feeds and less adoption of forage conservation techniques like hay and silage making. Human population in Ethiopia is rapidly increasing and this has a significant influence on agricultural land use as grazing lands are used for intensive crop production. This caused reduced availability of free feed resources from communal grazing lands.

Benishangul-Gumuz region is characterized by long rainy season (May-October) and 3% of its geographical area is covered by grasslands. In wet season of the year green forages are plentiful, but serious feed shortages occur in dry season. Thus, conservation of feeds produced in wet season is important to feed livestock in dry season. Green forages can be conserved as hay or silage. In case of Benishangul-Gumuz, grasses are at good nutritional quality to harvest when the rain is hard and this will wet the entire grasses which then leach and rots if hay making is practiced. When weather is good for sun-drying, grasses usually flower and lignify rapidly. Due to this reason, *Hypernia rufa* which is one of the most abundant grass species in grasslands of

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Benishangul-Gumuz is underutilized. The mismatch between weather and the plants physiology gives a comparative advantage for silage making than hay making.

The ensiling process is a preservation of moist forages under anaerobic conditions to enhance the nutrient content. This results decrease in pH and preserves moist forages from spoilage of microorganisms [1]. Silage making technique is safe and easy to use, does not pollute the environment and the products are natural [2]. However, it is not practiced among smallholder farmers in Benishangul-Gumuz region, but currently dairy is an emerging agricultural activity in the area. As a result, farmers and business men engaged in the dairy sector need sustainable feed resources for their animals, especially in dry season. Thus, making silage from abundantly found *Hypernia rufa* can be a good opportunity. However, the fermentation characteristics as well as the nutritional aspects of the grass have not been identified so far. Furthermore, an appropriate harvesting stage and influence of additives for making quality silage were not studied for this grass species. Therefore, this paper examines the effect of harvesting stage and additives on fermentative characteristics and nutritional quality of *Hypernia rufa* silage.

MATERIALS AND METHODS

The Study Area: The study was conducted at Assosa Agricultural Research Center which is located in Benshangul-Gumuz Regional State, Western Ethiopia. It is located10°30'N latitude and 034°20'E longitude, an altitude of 1565 meters above sea level. The pattern of rain is uni-modal with mean annual rainfall of 1275mm. The minimum temperature varies between 14°C and 20°C and the maximum temperature ranges from 25°C to 39°C. The soil type is reddish brown nitisols [3].

Treatments and Preparation of Grass for Silage: The source of plant material *(Hypernia rufa)* for the experiment was local pasturelands. Before cutting the plants for ensiling purpose, sample grass of known weight was oven dried at 63°C for 72 hours to determine the initial DM content of the herbage. Two factors considered in the experiment were harvesting stages and presence or absence and type of silage additives. Three harvesting stages were considered for ensiling the grass; early vegetative, boot and full bloom stage of growth. In each phase the grasses were cut at 10 cm above the ground and chopped in small sizes of 2-3cm for ensilage. PVC containers of 30L were used for ensiling purpose. The chopped grass materials at each stage were ensiled alone, with inclusion of 4% molasses, 1% urea and combination of 4% of molasses and 1% urea. The inclusion of both molasses and urea was based on fresh biomass basis. The molasses was mixed with hot water at 1:1 prior to adding it to chopped grass. The treatments are indicated below.

Treatment 1: Vegetative without additives (VWA)
Treatment 2: Vegetative with molasses (VM)
Treatment 3: Vegetative with urea (VU)
Treatment 4: Vegetative with molasses and urea (VMU)
Treatment 5: Boot without additives (BWA)
Treatment 6: Boot with molasses (BM)
Treatment 6: Boot with urea (BU)
Treatment 7: Boot with molasses and urea (BMU)
Treatment 8: Full bloom without additives (FBWA)
Treatment 9: Full bloom with molasses (FBM)
Treatment 10: Full bloom with urea (FBU)
Treatment 11: Full bloom with molasses (FBM)
Treatment 12: Full bloom with molasses and urea (FBMU)

Ensiling Procedure and Determination of Silage Quality: The containers were filled with mixtures and compacted in order to eliminate oxygen and sealed with tight cover to obtain sustainable anaerobic conditions. Each treatment was replicated three times having a total of 36 containers. Then the mini-silos (containers) were stored at room temperature (22°c) for 42 days. Thereafter, pH was determined from wet sample taken from each container immediately after opening on the 42^{nd} day of storage. The pH meter (model HI8521) used to measure the pH of the silage. The dry matter contents of the silage were determined by oven drying at 63°C for 72 hours. Ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and *in vitro* dry matter digestibility (IVDMD) were analyzed from dried silage samples. Ash and CP were analyzed according to AOAC [4] procedures. The NDF, ADF and lignin contents were analyzed according to Van Soest [5]. IVDMD was determined according to Tilley and Terry [6].

Statistical Analysis: The General Linear Model (GLM) procedure of statistical analysis system of SPSS (Version 20) for analysis of variance was used in analyzing data. The statistical model used was:

$$Yijk = \mu + Si + Aj + SAij + ijk$$

Where, $Yijk$ is dependent variable, μ is overall mean, Si is the fixed effect of harvesting stage, i=Early vegetative, boot and full bloom stage; Aj is the fixed effect of additives, j= without additives, molasses, urea and molasses + urea; *Saijk* is the interaction of harvesting stage i and type of silage additive j and *ijk* is a random error. Treatment means were separated by LSD.

RESULTS

The dry matter contents of grass before ensiling were 27.6, 30.9 and 37.7% for early vegetative, boot and full bloom stages, respectively. The DM content increased with increasing level of growing stage. The effect of harvesting stage and additives on chemical composition and quality of silage from *Hypernia rufa* is presented in Table 1. Both harvesting stage and type of additives had a significant effect ($P<0.01$) on DM content of silage. The DM content was significantly higher in full bloom than boot and early vegetative stages. In the same manner, DM in boot stage is significantly higher than early vegetative stage. The DM content was significantly higher in silages with molasses plus urea and with molasses compared to silages without additives and with urea.

PH was significantly affected ($P<0.001$) by type of additives, but not by the harvesting stage. The lowest pH which indicates the desirable fermentation process was attained in silages with molasses. On the contrary, the highest pH was recorded in silages with urea. Silage without additive showed lower pH than silages with urea and silages with urea plus molasses. The CP content was significantly affected by both harvesting stage and type of additives with significant interaction. The highest CP content was attained at early vegetative stage followed by full bloom; and the lowest CP recorded at boot stage. Silages with urea had higher CP content than silages with molasses and molasses plus urea.

Higher amount of ash which is undesirable was recorded irrespective of harvesting stage and type of additives. NDF, ADF and IVDMD were significantly affected ($P<0.001$) by harvesting stage, but not by the type of additives. Hypernia silage from early vegetative stage had significantly lower NDF and ADF than boot and full bloom stages. Accordingly, silage from early vegetative stage had significantly higher IVDMD than boot and full bloom stages.

The interaction of harvesting stage and type of additives on silage chemical composition and quality parameters is presented in Table 1. DM recorded in VU was significantly lower than others. PH was the highest in VU, BU and FBU, whereas the lowest pH was recorded in VM, BM and FBM. CP was significantly higher in VM and VMU than others. The lowest CP was recorded in BM, BMU and FBMU.

Table 1: Effects of harvesting stage, additives and their interaction on chemical composition and fermentative characteristics of *Hypernia rufa* silage

Harvesting stage	DM	pH	CP	Ash	NDF	ADF	Lignin	IVDMD
Early vegetative (V)	28.9^{c}	6.6	6.7^{a}	ND	64.4^{c}	37.9^{b}	ND	57.1^{a}
Boot (B)	31.6^{b}	6.5	3.8^{c}	11.6	68.4^{a}	45.5^{a}	5.0	55.7^{b}
Full bloom (FB)	33.9^{a}	6.4	4.1^{b}	11.6	68.7^{a}	47.3^{a}	5.0	55.6^{b}
SE	0.63	0.09	0.09	0.07	0.226	0.721	0.06	0.267
P-Value	0.000	0.282	0.000	0.398	0.000	0.000	0.245	0.022
Additives								
Without additives (WA)	30.3^{b}	6.4^{c}	4.9^{ab}	11.7	67.2	43.0	5.1	56.1
Molasses (M)	32.4^{a}	4.5^{d}	4.8^{b}	11.5	66.9	44.0	4.9	56.0
Urea (U)	29.5^{b}	8.2^{a}	5.1^{a}	11.5	67.3	43.9	5.0	55.9
Molasses +Urea (MU)	33.5^{a}	6.9^{b}	4.7^{b}	11.5	67.2	43.4	4.9	56.0
SE	0.73	0.11	0.11	0.10	0.59	0.83	0.09	0.39
P-Value	0.002	0.000	0.029	0.606	0.958	0.837	0.676	0.252
Harvesting stage * Additives								
VWA	27.1^{b}	6.5^{c}	6.3^{b}	-	64.2^{b}	38.2^{c}	-	57.3^{a}
VM	31.8^{a}	4.7^{d}	7.1^{a}	-	63.7^{b}	37.6^{c}	-	57.1^{a}
VU	26.0^{c}	8.1^{a}	6.5^{b}	-	65.2^{b}	38.2^{c}	-	56.6^{a}
VMU	34.67^{a}	7.2^{b}	7.0^{a}	-	64.6^{b}	37.7^{c}	-	57.5^{a}
BWA	29.8^{b}	6.5^{c}	4.1^{cde}	11.5	68.6^{a}	46.2^{ab}	5.3	56.0^{a}
BM	31.9^{a}	4.5^{d}	3.4^{f}	11.7	68.6^{a}	46.2^{ab}	4.9	55.2^{b}
BU	29.7^{b}	8.5^{a}	4.4^{cd}	11.6	67.9^{a}	45.6^{ab}	5.0	55.9^{ab}
BMU	35.1^{a}	6.5^{c}	3.4^{f}	11.4	68.4^{a}	44.1^{b}	4.9	55.2^{b}
FBWA	34.0^{a}	6.2^{c}	4.3^{cd}	11.9	68.9^{a}	44.6^{ab}	5.0	55.0^{b}
FBM	33.5^{a}	4.4^{d}	4.0^{de}	11.6	68.5^{a}	48.1^{ab}	4.9	55.6^{b}
FBU	32.8^{a}	7.9^{a}	4.6^{c}	11.5	68.9^{a}	47.9^{ab}	5.0	55.1^{b}
FBMU	35.1^{a}	7.1^{b}	3.6^{ef}	11.6	68.5^{a}	48.5^{a}	5.0	55.3^{b}
SE	1.26	0.19	0.19	0.15	1.03	1.44	0.12	0.75

ND= Not Determined, DM= Dry matter, CP=Crude protein, NDF= Neutral Detergent Fiber, ADF= Acid Detergent Fiber, IVDMD= *in vitro* dry matter digestibility; means with different letters in a column are significantly different at P=0.05

DISCUSSION

The increase in DM of silage with maturation of hypernia grass could be attributed to increased cell wall contents and decreased moisture content. Hypernia silage with molasses had significantly higher DM content which could be attributed to fast fermentation process due to molasses. Seglar)[7] observed that the faster the fermentation is completed, the more nutrients are retained in silage. This is due to the fact that cellulolytic microbes could not strive under acidic conditions resulting in no microbes reducing DM. This finding is in agreement with Yokota *et al*. [8] and Yunus *et al*., [9], who reported that molasses increases the DM of silage. Bilal [10] also reported that the DM recovery was higher due to additives compared to control in silage of mott grass.

Addition of molasses to silages in numerous experiments has been to be effective silage additive in terms of promoting lactic acid fermentation, reducing silage pH, discouraging a clostridial fermentation and proteolysis and generally decreasing organic matter losses. Molasses provides sugars as a substrate for lactic acid bacteria that increases accumulation of lactic acid resulting in low pH. As indicated by Falola *et al*., [11], pH is the one of the simplest and quickest ways of evaluating silage quality which is influenced by the moisture content and the buffering capacity of the original materials. A good quality grass and legume silage-pH in tropics ranges between 4.3 and 4.7 [12]. Thus, silage with molasses in this experiment has shown pH of 4.5 which is of good quality.

The higher pH recorded in silage with urea is related with its higher buffering capacity. Studies indicated that corn forage may have a pH of 5.9 but urea treated corn forage will have a pH of about 8.5 to 9.0 [13]. Other studies also reported that addition of urea or urea plus molasses into corn silage increased silage pH [14, 15]. High pH in hypernia grass silage without any additive may be attributed to low concentration of fermentable carbohydrates in the original grass.

The higher CP content in early vegetative stage indicates that cutting of hypernia grasses has to take place at the early stage of growth otherwise protein decreases rapidly as lignification proceeds. This finding is in agreement with Titterton and Bareeba [16] as they reported that protein and digestibility both decline rapidly after flowering in tropical grasses. Bolsen [17] also reported that quality silage can be obtained from tropical grasses if they are cut at early vegetative stage and molasses is added. In this experiment, significantly higher content of CP was recorded in silages from hypernia grass harvested at early vegetative stage and treated with molasses and molasses plus urea. The possible reason behind increase in CP during ensiling may be due to proteolytic activity during fermentation which produces NH_3. But, due to efficient fermentation in early stability of silage, this proteolysis activity is inhibited and the produced NH_3 helps in getting the aerobic stability because of its fungicidal properties [18]. The CP value (3.4-7.1 g/100g) for the present study was lower than the critical of 7.7% recommended for small ruminants and 10-12% for ruminants NRC [19]. This indicates that CP is limited in hypernia grass silage and therefore suggests a supplementation with richer protein sources. Addition of urea to hypernia silage could be an economical source of protein. However, in this study it had an adverse effect on fermentation quality of silage though Sarwatt [20] obtained good silage by applying 0.5% urea to maize, sorghum and Rhodes grass in Tanzania.

A high level of ash recorded in this study indicates contamination by soil, which is indigestible. Naseri [21] reported that over 10% ash in silage or concentrate is an indication of soil contamination. Thus, proper handling is important during ensiling process. The increasing level of NDF and ADF with grass maturity is attributed to increasing percent of cell wall material. NDF and ADF contents are inverse predictors of intake and digestibility, respectively. Thus, improving NDF and ADF content of silage by harvesting grasses at early stage can contribute to animal feeding by increasing DM intake and digestibility. This finding is in agreement with Odedire [22] who noted a rapid decline in the DM intake and digestibility of eight week old *Andropogon gayanus* fed WAD goats than those on 4 and 6 weeks old. Digestibility can be used as indication for feed quality. According to Naseri [21], digestibility of over 70%, 60-70%, 40-60% and under 40 % are categorized as good, moderate, low and very low digestibility, respectively. IVDMD of hypernia silage in present study is between 55% and 57.5% so that it's low in digestibility. This could be associated with low digestibility of hypernia grass. Loures *et al*. [23] indicated that the final quality of silage is directly linked to the material that originated it. The main interest of this experiment, however, is not to improve the feed quality of hypernia grass; rather it's to make the grass available year round. This grass is a feed of low cost and high availability in Benishangul-Gumuz, but not available in dry seasons as it lignifies rapidly.

CONCLUSION

Harvesting *Hypernia rufa* at an early growth stage would improve crude protein content and digestibility of silage. Use of molasses as silage additive when economically feasible would increase the concentrations of DM and fermentable substrates and, thereby, improve fermentation characteristics. Thus, appropriate silage can be made from *Hypernia rufa* by harvesting it at early vegetative stage and adding molasses at 4% on fresh biomass basis. Consequently, this could improve the green feed availability of the area in dry season. However, researches should be done further on determination of optimum post-ensiling period with good silage fermentative characteristics.

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