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Research Paper

Effect of fermented juice of epiphytic lactic acid bacteria and molasses on *Brachiaria obtusiflora* silage fermentation quality and milk production in Nubian goats

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ABSTRACT
The aims of the present study was to: (i) evaluate the effect of fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses on the nutritive value of Brachiaria obtusiflora silage, (ii) evaluate the feed intake and digestibility of goats fed Brachiaria obtusiflorasilage treated with FJLB, molasses or combination (iii) determine milk yield and composition of goats fed Brachiaria obtusiflorasilage treated with FJLB, molasses or combination. The Brachiaria obtusifloragrown in University of Bahri farm was harvested after 60 days of planting. The grass was chopped into 2- to 3-cm length and mixed with silage additives. The treatments were: untreated silage (US), silage prepared with 5% molasses (MO), 1% FJLB (FJLB) or 5% molasses plus 1% FJLB (MO+FJLB). After ensiling for 45 days, four groups of lactating Nubian goats (8 each) were divided randomly and assigned to the one of the dietary treatments; US, MO, FJLB or MO+FJLB for 90 days. The results showed that the dry matter contents of the Brachiaria obtusiflora silage was improved (P<0.05) when treated with additives (MO, FJLB and MO-FJLB) compared the control group. Crude protein content was higher (P<0.05) in the group treated with MO+FJLB compared to the control group. The neutral detergent fiber contents of Brachiaria obtusiflorasilage was lower (P<0.05) in the group treated with MO and MO+FJLB compared to the control while the acid detergent was not affected. Moreover, the intake of organic matter, crude protein and nitrogen free extract was superior (P<0.05) to the group received MO+FJLB. However, the nutrient digestibility was not affected by dietary treatments, except for crude protein which was higher (P<0.05) in MO+FJLB group compare to US group. Milk yield was significantly higher (P<0.05) in the groups received MO and MO+FJLB compared to other two groups. Milk protein content was higher (P<0.05) in the both groups received MO+FJLB and FJLB compared to US, otherwise, no significant differences were observed among the groups in other parameters. In conclusion, treatment of Brachiaria obtusiflora silage with MO+FJLB was the best choice to improve the nutrient value of the grass silage and goat's performance.
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I. INTRODUCTION

Limited water availability during dry season is major constraint for range land grass production in Sudan. Conservation of grasses as silage could be an alternative to overcome the problems livestock feed shortage Conservation of grasses as silage is always hindered by poor quality products during the process of fermentation mainly due to the lack of WSC and LAB. Fermented epiphytic lactic acid bacteria and molasses addition could improve the silage quality which in turn will affect the animal performance in terms of quantity and quality of the products. Most of the previous studies have been conducted in experimental silos which suggest a need to evaluate these grasses silage in trails with animals. Understanding the animal response to feeding diets based on grass silages, in terms of quantity and quality of the products, is essential to find more efficient techniques of grass-silage production and the correct balance of these diets. The *Brachiaria obtusiflora* has been chosen for the present study because it is characterized by tolerance to the high solidity soil such as that found in the research location area.

Milk quality is of specific concern in goat milk production. The fatty acid composition of milk may influence its nutritive value and health value for consumers (Mensink et al., 2003). Forages form a major part of ruminant diets in most farming systems, and sometimes contain significant amounts of fat and polyunsaturated fatty acids (PUFA). Diets based on pasture and grass silages can thus improve the nutritional quality of milk and meat by shifting their FA composition towards less saturated FA and more PUFA, especially omega-3 FA (Dewhurst et al., 2006). However, evaluation of additive added *Brachiaria obtusiflora* silage as feed for dairy goats has been little studied, and its potential to improve desired PUFA in milk is unknown.

Sudan's livestock population has been estimated to be around 100 million heads (MARF, 2011) but it has not yet achieved its potential in milk production. Poor milk production, especially from goats, is due to the poor nutrition which is related to seasonal variation in forage availability and quality. In Sudan, most of the forage production is occurs in the rainy season. Forage conservation could be an alternative to overcome the forage deficit problem during the dry season. Silage is forage conservation method most used and corn and sorghum are the most used crops for this process because they have high yields and are specific for the good quality silage produced.

Brachiaria obtusiflora is a tropical grass grown widely in many areas of the world. It is used to feed livestock, as well as adapted to drought conditions (Bureenok et al., 2011). However, its low productivity during the dry season limits animal production. Tropical forage crops can be conserved as silage, but they are known to be difficult to ensile, and resulting fermentation quality, intake and digestibility are quite low (Niimi and Kawamura, 1998). Good quality silage requires epiphytic lactic acid bacteria (LAB) and water-soluble carbohydrate (WSC) to produce sufficient lactic acid for rapid pH reduction (McDonald et al., 2010). To improve the quality of fermentation, it is important to find suitable additives, presently; natural additives are preferred. Therefore, the fermented juice of epiphytic lactic acid bacteria (FJLAB) has been recommended as a silage additive for tropical grass silage (Bureenok et al., 2005; Bureenok et al., 2005a; Bureenok et al., 2011). Previous reports have suggested that the addition of molasses as source of readily fermented WSC increased lactic acid bacteria and feeding quality of tropical grass silage (Umana et al., 1991; Yunus et al., 2000; Van Neikerk et al., 2007).The objective of the present study was to evaluate the effect of fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses on nutrient intake and digestibility, milk yield and composition in Nubian goats.

II. MATERIALS AND METHODS

Study location

The experiments were conducted during the season 2016/2017 at the College of Animal Production Farm; University of Bahri campus at about 1.5 km east at El Kadaru area in the northern part of Bahri. The campus is situated about 19 km north of Bahri between latitudes 15° 45' 13.27" North and longitudes 32° 35' 40.96" East. It is around 22 km north of downtown Khartoum at altitude 398 meters above sea level (a.s.l.). The experimental field is 2.47 ha of predominantly heavy clay, with an initial pH of 8.5 (Mohammed, 2012). Soil analysis of the University of Bahri farm indicated that, the soil is heavy clay with pH of 7.99-8.37, soil organic carbon 0.09-0.15% and soil nutrients (mmol+/1) Ca 2.5-15.25, Mg 2.5-15.25, K 0.06-0.18, Na 3.6-89.0, (%) N 0.02-0.037 and (mg) P 2.7-3.8 (Mohammed, 2012).

Land preparation

Deep ploughing during field preparation by disc plough followed by twice disc harrowing and there after cultivator with levelers. A fertilizer containing 40 kg N/ha, 17 kg P/ha and 66 kg K/ha was applied to stimulate growth of *Brachiaria obtusiflora* after preparation, 10 kg/ha *Brachiaria obtusiflora* seeds was spread in the soil manually. Immediately after sowing, and every week thereafter; water was pleated to cover all plots.

Preparation fermented juice of lactic acid

FJLB was prepared from 200 g fresh *Brachiaria obtusiflora* grass by macerating in 1000 ml of sterilized distilled water with a home blender. The juice was filtered through double layer of cheesecloth; the juice was transferred into a glass bottle and 2% of glucose will be added. The bottle was caped and incubated in room temperature for 2 days.

Silage preparation

Brachiaria obtusiflora grown in the experimental field, University of Bahri, Sudan, was harvested after 60 days of planting. The grass was chopped with a forage cutter into 2- to 3- cm lengths and mixed with silage additives. The silages being untreated (US), or prepared with 5% molasses (MO), 1% FILB (FJLB) or 5% molasses plus 1% FJLB (MO-FJLB). FJLB was applied at 1% of fresh weight (at log 5.64 cfu/g fresh weight). Molasses (70% dry matter (DM), 1% crude protein (CP) and 24% WSC on DM basis) was applied at 5% fresh weight. The combined additive (MO-FJLB) was mixed thoroughly and sprayed on the silage materials. To

adjust the moisture content of the MO and CO, (1% fresh matter) was added. All the mixtures were packed in 100 kg plastic drums and stored at room temperature for 45 days. Three samples per treatment were subjected to chemical analysis.

Feed sampling and analysis

Representative samples of silage and feed was collected and stored at -20 ºC until analysis. All samples were subjected to chemical analysis by the procedure described in AOAC(1990).

Does feeding

Thirty-two, approximately one-year old, Nubian goats were randomly assigned to one of the four dietary treatments after one week of kidding in completely randomized block experiment. The dietary treatments were untreated (US), or silage prepared with 5% molasses (MO), 1% FILB (FJLB) or 5% molasses plus 1% FJLB (MO-FJLB). Does were individually housed in soil-surfaced pens under shade, without bedding and with free access to water. Experimental diets were offered to the does individually at 0800 and 1600 h in two equal portions. The experimental period lasted 90 days; 15 days of adaptation to the new diet, and 75 days for measurements (feed intake, milk yield) and sample collection (sampling of feed and orts and milk). Milk composition was measured by using the milk analyzer "Lactoscan SP".

Nutrient digestibility and chemical analysis

A total of 4 lactating Nubian does were randomly assigned to four experimental treatments in a Latin square design, with four treatments, four periods and four does per treatment within each period, resulting in 4 replicates per treatment. The four experimental treatments were assigned randomly to the four groups in the first period, after which a predetermined sequence was followed that allowed each doe to receive each treatment.During the collection period, feed intake was recorded daily. Fecal outputs were collected twice daily from each doe at 0700 and 1500 h and stored at −10°C for later analysis. About 100 g (as-is basis) of feces from each doe was taken daily and pooled within each period. The composited samples were dried in a forced-air oven at 65°C for 72 h, ground along with feed and orts samples to pass a 1-mm screen using a Wiley mill grinder, and retained for compositional analyses. Feed, orts and fecal samples were analysed for DM, ash, nitrogen, ether extract (EE) and ADF according to AOAC (1991) official methods. Neutral detergent fiber (without the use of alpha amylase but with sodium sulphite) and lignin (by solubilization of cellulose with sulphuric acid in the ADF residue) were determined by the procedure of Van Soest et al. (1991).
Milk samples were tested for fat, SNF, lactose and protein contents using milk analyzer (Lactoscan LA, Bulgaria).

Statistical analysis

Nutritive value data was subjected to one-way analysis of variance using general linear model of SAS (SAS, 2003) as completely randomized block design. The Duncan multiple range test was used to further compare means at $P<0.05$.

III. RESULTS AND DISCUSSION

Nutrient content, intake and digestibility of brachiaria silage:

The effect of adding M, FJLB and their combination is presented in (table 1). The results showed that the dry matter and ash contents of silage were significantly reduced when the MO-FJLB combination inoculated. This can be attributed to enhancement of microbial activity in the silos which resulted in more consumption of the dry matter. Additionally, the crude protein content was improved in the group treated with MO-FJLB. Inoculation of MO-FJLB made avail source of water-soluble carbohydrate (WSC) for bacteria in the silo which induced to consume it instead of protein in the silo. Moreover, the dead bacteria are also counted in the analysis of crude protein content of the silage. The fiber content of the silage is also reduced in the treatment MO-FJLB compared to other groups. Reduced NDF content in ensiled materials inoculated with M, FJLB or combination compared to the US, can be illustrated by microbial degradation of carbohydrates released from disrupted lignin-carbohydrates complexes during ensiling (Khalilvandi et al., 2010, Van Soest, 1994).

Organic matter intake, crude protein intake, nitrogen free extract intake and crude protein digestibility was superior in the group of goats received the FJLB+MO compared to those received the control diet (table 2) indicating that the inoculation of molasses and fermented juice of lactic acid bacteria tobrachiaria silage improved the utilization of these nutrients.

The greater apparent fecal digestibility of CP after the feeding of the rations containing silage treated with M+FJLB corroborates data by Mustafa *et al*. (2000) who demonstrated that CP originating from inoculated silages have a higher apparent digestibility compared to that of grasses. However, the underlying reason of the higher apparent CP digestibility is not exactly clear butit might be related to the level of CP intake. It can be

speculated that, at least in part, the contribution of endogenous CP in feces was relatively lower at higher CP intakes resulting in a somewhat higher apparent digestibility of CP (Bureenok et al., 2016).

Milk yield and composition

Milk yield and composition is presented in (figure 1 and table 3) respectively. Milk yield was superior in the group fed MO and MO+FJLB compared to the US and FJLB group. Molasses has been proven to be an effective silage additive and it can be added up to 10% w/w to provide fast fermentable carbohydrate for the ensilage of tropical herbages (Yitbarek and Tamir, 2014). Similar finding was reported by Mandal et al. (2001) and Shatrughan Shah et al., (2018), who found a positive correlation between molasses and milk yield in Buffalo.

IV. CONCLUSION

Inoculation of *Brachiaria obtusiflora* silage with different additives improved the nutritive value of the grass. MO-FJLB is recommended to be inoculated when making silage from tropical grass. More research is required to test the effect of silage additives (MO, FJLB and combination) on nutrient digestibility in vivo and milk yield and composition in Nubian goats. Carcass characteristics and meat quality of the kids should be tested also.

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Table1. Effect of fermented juice of lactic acid bacteria and molasseson *BrachiariaObtusiflora* silage nutrient contents

	Treatments					
	US	**MO**	**FJLB**	**MO-FJLB**	**SE**	**P-value**
DM	97.5[a]	96.17[ab]	96.20[ab]	96.93[b]	0.19	0.04
Ash	13.45[a]	13.15[a]	12.65[b]	11.92[c]	0.08	0.001
CP	9.43[c]	10.74[b]	9.14[c]	11.59[a]	0.073	<0.0001
EE	1.22[b]	1.01[b]	0.82[b]	2.07[a]	0.10	0.004
ADF	41.04	41.08	42.5	40.55	0.58	0.247
NDF	72.50[a]	67.00[b]	68.00[ab]	64.60[b]	0.81	0.01
ADL	11.00[a]	7.50[ab]	4.50[b]	6.50[ab]	0.97	0.037

US, Untreated silage; MO, 0.1% Molasses; FJLB, Fermented Juice of Lactic Acid Bacteria; DM, Dry Matter; CP, Crude Protein; EE, Ether Extract; ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; ADL, Acid Detergent Lignin.
SE, Standard error.
[a,b,c,] means sharing same letters in the same raw are significantly differ.

Table 2. Effect of fermented juice of lactic acid bacteria and molasses on *Brachiaria Obtusiflora* silage nutrient contents intake and digestibility

	Treatments					
Parameter	**UN**	**FJLB**	**MO**	**MO+FJLB**	**SE**	**P VALUE**
Nutrient intake kg/day						
DM	1.13	1.19	1.12	1.30	0.04	0.06
OM	1.01[ab]	1.06[ab]	1.00[b]	1.19[a]	0.05	0.04
CP	0.15[b]	0.18[ab]	0.16[b]	0.20[a]	0.01	0.002
EE	0.03	0.03	0.03	0.06	0.01	0.12
CF	0.22	0.22	0.22	0.23	0.01	0.73
NFE	0.54[ab]	0.55[ab]	0.52[b]	0.61[a]	0.02	0.05
Nutrient digestibility %						
DM	74.47	73.33	72.92	78.75	2.74	0.45
OM	74.96	73.40	76.09	79.27	2.91	0.55
CP	78.45[b]	81.86[ab]	79.61[ab]	86.40[a]	1.66	0.03
EE	83.19	84.54	78.09	87.26	2.83	0.19
CF	52.31	44.91	56.07	59.80	5.15	0.26
NFE	82.39	80.40	83.84	83.17	2.71	0.82

US, Untreated silage; MO, 0.1% Molasses; FJLB, Fermented Juice of Lactic Acid Bacteria; DM, Dry Matter; CP, Crude Protein; EE, Ether Extract; CF, Crude Fiber; NFE, Nitrogen free extract
SE, Standard error.[a,b,,]means sharing same letters in the same raw are significantly differ.

Table 3.Effect of*Brachiaria Obtusiflora*silage treatment with fermented juice of lactic acid bacteria and molasses on goat's milk contents

	Treatments					P VALUE		
Parameters	**UN**	**FJLB**	**MO**	**MO+FJLB**	**SE**	**Treatment**	**Time**	**Treatment*time**
Freezing point	-0.40[a]	-0.42[ab]	-0.41[a]	-0.43[b]	0.01	0.01	0.90	0.056
Salt	0.53[b]	0.56[ab]	0.54[b]	0.57[a]	0.01	0.01	0.41	0.10
pH	6.59	6.62	6.68	6.60	0.02	0.05	0.54	0.66
Lactose	4.36[b]	4.48[ab]	4.41[ab]	4.59[a]	0.05	0.01	0.49	0.69
Water content	86.76	86.59	86.73	86.22	0.4	0.54	0.06	0.88
Temperature	18.07	18.43	18.99	17.59	0.50	0.14	0.01	0.01
Fat	4.84	4.73	4.80	4.66	0.30	0.01	0.93	0.34
SNF	8.35	8.68	8.47	8.79	0.10	0.01	0.42	0.13
Protein	3.46[b]	3.65[a]	3.52[ab]	3.66[a]	0.04	0.01	0.31	0.01

US, Untreated silage; MO, 0.1% Molasses; FJLB, Fermented Juice of Lactic Acid Bacteria; DM, Dry Matter; CP, Crude Protein; EE, Ether Extract; CF, Crude Fiber; NFE, Nitrogen free extract
SE, Standard error.[a,b,,]means sharing same letters in the same raw are significantly differ.