Research Article

Influence of Liquid Whey and Molasses on Chemical Composition and Fermentation Characteristics of Reed Plant Silage

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Abstract | This study was conducted to evaluate the effects of adding liquid whey (LW) and molasses (M) on the chemical composition and fermentation characteristics of reed plant silage (RS). Liquid whey was added to introduce lactic acid bacteria (LAB) at three levels: 0 (0LW), low (LLW), and high (HLW). Molasses was added at levels of 4%, 6%, and 8%. The reed plant, with the respective additives, was ensiled in plastic bags for 60 days. Results showed that dry matter (DM) and ether extract (EE) contents significantly increased ($p < 0.01$) in both LLW and HLW treatments. Crude fiber (CF) content decreased ($p < 0.01$) with each increase in LW level, while the highest crude protein (CP) content ($p < 0.05$) was observed in the HLW treatment. Increasing molasses levels led to significant increases in DM, organic matter (OM), and nitrogen-free extract (NFE) contents, and a decrease in CF content ($p < 0.01$). Higher molasses levels also resulted in significantly greater CP and EE contents ($p < 0.01$). The addition of LLW improved the fermentation quality of RS, as evidenced by lower ($p < 0.01$) pH, residual water-soluble carbohydrates (WSC), and ammonia nitrogen (NH_3-N), along with higher ($p < 0.01$) concentrations of lactic acid (LA) and volatile fatty acids (VFA), compared to RS prepared without LW. Further improvements ($p < 0.01$) were observed with the addition of HLW. Increasing molasses levels from 4% to 6% and 8% significantly decreased ($p < 0.01$) pH, residual WSC, and NH_3-N contents, while LA and VFA concentrations increased ($p < 0.01$ and $p < 0.05$, respectively). The chemical composition and fermentation quality of RS were also significantly affected by the interaction between LW and M levels. It was concluded that high-quality reed plant silage can be produced by incorporating both liquid whey and molasses, improving both chemical composition and fermentation characteristics.

Keywords | Liquid whey, Molasses, Fermentation, Lactic acid bacteria, Silage, Wild reed

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INTRODUCTION

Agricultural by-products are used as the main feed for ruminant due to their availability and low prices. Ruminants would be more dependent on these materials as a result of the continuous increase in the population and competition for grain and related by-products used in poultry diets. These feeds are generally characterized with low nutritive value due to low crude protein and high structural carbohydrates contents resulting in lower digestibility and animal performance (Khan *et al*., 2015; Wizna *et al*., 2025). Saeed *et al*. (2021) reported that there is a need to improve the utilization of locally available feed ingredient to enhance animal performance together with reducing production cost and minimizing protein and energy deficiency.

Reed plant, *Phragmitis communis*, due to its wide spread in various soils, may be considered one of the basic and cheapest feed sources for ruminants (Beyzi *et al*., 2023). However, effective introduction of such a natural plant into feeding systems of ruminants requires the use of some treatments to increase palatability and enhance its chemical composition. Ensiling is an effective method for preserving the nutritional value of forages. In many regions, silage is a major source of feed for ruminants (Wilkinson and Rinne, 2018).

Studies indicate that the reed plant has high structural fibers and low WSC content, which hinders anaerobic fermentation, and makes it difficult to achieve rapid pH decrease (Kazemi *et al*., 2024). Al-Sultani (2016) concluded that ensiling reed plant with molasses and urea may produce good quality feed. Beyzi *et al*. (2023) reported that using whey as a source of LAB and fermentable nutrients are a promising strategy for improving reed plant fermentation. Liu *et al*. (2025) confirmed that adding molasses at a level of 10% DM of reed plant significantly improved the quality of silage.

In Iraq the liquid whey a by-product of cheese industry is normally thrown away and often dumped with sewage water, posing a pollution problem and public health risk. Addition of liquid whey as a source of lactic acid bacteria (LAB) in grass silage could improve the fermentation pattern and may lessen the environmental impact (Santos *et al*., 2006). Molasses a byproduct of sugar industry is a common fermentation stimulant used to encourage the growth of LAB (Gao *et al*., 2021). Bai *et al*. (2024) justified addition of molasses to improve the fermentation process since LAB does not have the ability to ferment starch. Bautista-Trujillo *et al*. (2009) emphasized the feasibility of using the whey and molasses for maize plants silage. However, little is known about the potential of these additives to ensile reed plant. Therefore, this study aimed to evaluate the effect of adding different levels of both liquid whey as a natural source of LAB and molasses required by this microorganism as a substrate on chemical composition and fermentation of reed plant silage.

MATERIALS AND METHODS

Preparation of reed plant silages

Samples of reed plant silages (RS, 500-600 g) were prepared in a laboratory. Reed plants (RP, 50.08% DM at harvest) were chopped into 1.5-2 cm of length. Liquid whey (LW) obtained from local Animal breeder, was screened for LAB and added at ensiling to ensure introducing LAB to RP at 0 (without LW, 0LW), low (LLW) and high (HLW) levels (1×10^5 and 1×10^6 cfu/g FM, respectively). Number of LAB in LW was counted on the deMan, Rogosa, and Sharpe agar prepared and sterilized according to the manufacturer's instructions after incubation anaerobically at 37°C for 48 h (Addaha *et al*., 2014). Cane molasses (M) obtained from the Etihad Food Industries Co. Ltd. in Babylon Province was added as a source of water-soluble carbohydrates (WSC) at levels of 4, 6 and 8%. Urea was added as a source of nitrogen (N) at 1%. Molasses and urea were added on DM basis of RP. The amounts of LW added were estimated based on LAB content (3.5×10^6 cfu/ml), ensuring both LLW and HLW levels. These amounts were included in the amount of water used to dilute molasses, dissolve urea, and reduce the DM level of the ensiled materials to 35%. Accordingly, nine RS treatments were prepared with 5 replicates per each, and the total number of RS samples was 45. Samples of RS were packed in double nylon sacs, well compacted to remove the air, sealed and kept in pit silos for a period 60 days, at the end of this period samples were opened to determine the chemical composition and fermentation.

Chemical composition

Chemical composition was performed according to AOAC (2005) methods. Dry matter (DM) was determined by drying samples in an oven at 105 ºC for 24 hours (h). Dried samples were a grind and kept in plastic containers. Organic matter (OM) was determined by burning dried samples in furnace at 500 ºC for 4 h. Crude protein (CP) was determined using S4 Kjeltec system. Ether Extract (EE) was determined by extraction with hexane in Soxhlet apparatus. Crude fiber (CF) was determined using DOSI-Fiber Extractor. Nitrogen free extract (NFE) was estimated by difference. Chemical composition of RP and additives is shown in Table 1.

Table 1: Chemical composition of reed plant (%) on DM basis.

Nutrients[1]	Reed plant	Liquid whey	Molasses	Urea
DM	50.58	-	68.75	-
OM	84.42	-	87.77	-
CP	9.03	1.64	2.20	287.5 *
EE	3.29	-	0.69	-
CF	47.82	-	0.10	-
NFE	24.1	-	84.78	-

[1]DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; CF: crude fiber; NFE: nitrogen free extract. *46 × 6.25.

Characteristics of silage fermentation

Silage fermentation characteristics were determined on the water extract prepared by mixing 50 g of each RS samples with 500 ml of distilled water in a blender for 10 minutes; the liquid was filtered through two layers of cheesecloth

(Levital *et al*., 2009). The pH of the RS was recorded in aqueous extract immediately before acidification using Mi 180 Bench pH Meter. The concentration of LA was determined using the colorimetric method described by Borshchevskaya et. al. (2016). The concentration of VFA was determined using the method proposed by Markham (1942). The concentration of residual WSC was determined using the colorimetric method described by Dubois *et al.* (1956). The concentration of NH_3-N was determined using the MgO distillation method (AOAC, 2005). Data obtained were analyzed as a factorial experiment in completely randomized design by analysis of variance using statistical analysis system (SAS, 2010).

RESULTS AND DISCUSSION

Chemical composition

Table 2 shows the chemical composition of reed plant silages (RS) as affected by adding different levels of LW and M. The results revealed that adding LW at low and high levels intended to introduce LAB at 1×10^5 and 1×10^6 cfu/g FM significantly (p<0.01) increased the DM content from 28.69% in RS samples prepared without LW to 29.76 and 30.01% respectively. Fallah (2019) observed that addition of fresh whey to whole plant corn at ensiling increased (p<0.05) DM content from 30.4 to 32.98%. However, no significant changes in DM content were shown in temperate grasses ensiled with different levels of fresh whey though there was a slight increase (Cajarville *et al*., 2012).

Table 2: Main effect of liquid whey and molasses levels on chemical composition of reed plant silages (mean ± SE).

Items	liquid whey (LW), cfu/g FM			Molasses (M), %			P	
	0	low	high	4	6	8	LW	M
DM	28.69^b ± 0.20	29.76^a ± 0.26	30.01^a ± 0.16	29.28^b ± 0.24	29.30^b ± 0.24	29.88^a ± 0.26	**	*
OM	79.38^b ± 0.20	79.45^b ± 0.30	80.89^a ± 0.25	79.20^c ± 0.20	79.72^b ± 0.32	80.80^a ± 0.25	**	**
CP	9.23^b ± 0.16	9.40^{ab} ± 0.19	9.84^a ± 0.25	8.83^b ± 0.20	9.66^a ± 0.11	9.98^a ± 0.19	*	**
EE	3.05^b ± 0.10	3.39^a ± 0.09	3.51^a ± 0.09	2.99^b ± 0.10	3.48^a ± 0.09	3.48^a ± 0.08	**	**
CF	45.88^a ± 0.46	43.79^b ± 0.45	42.23^c ± 0.43	45.13^a ± 0.58	43.95^b ± 0.58	42.82^c ± 0.46	**	**
NFE	21.21^c ± 0.50	22.86^b ± 0.63	25.30^a ± 0.41	22.24^b ± 0.43	22.63^b ± 0.60	24.51^a ± 0.62	**	**

Means with different superscripts in a row differ significantly at: * (p<0.05), ** (p<0.01); CFU: colony forming units; FM: fresh matter; LW: liquid whey; M: molasses; DM: dry matter; OM: Organic matter; CP: crude protein; EE: ether extract; CF: crude fiber; NFE: nitrogen free extract.

The increase in DM content may be due to the improvement of the fermentation resulting from the increased numbers of LAB, which lowers the pH and inhibits the activity of other microorganisms, thus avoiding DM loss. Bai *et al.* (2024) reported that the improvement of fermentation minimizes DM loss. Repetto *et al.* (2011) reported that fresh whey can be useful as an additive for silage production at up to 5% of fresh whey, because at this level, improvements of silage quality were maximized, and losses of nutrients during fermentation were minimized.

The results showed that the OM content was increased (p<0.01) in association with the addition of LW and its increased level. The mean values were 79.45 and 80.89% in the samples of RS prepared with low and high LW levels respectively, compared to 79.38% in those prepared without LW. Castaño and Villa (2017) observed a significant increase (p<0.01) in the OM content of Cuba grass ensiled with LW. Bautista-Trujillo *et al.* (2009) confirmed that adding LW or LW with M at ensiling of whole maize plant (stem and leaves) significantly (p<0.05) increased the OM content. This improvement in OM content may be due to whey content of protein and amino acids, which may stimulate growth of LAB, leading to higher stability of organic matter (Zhang *et al*., 2000).

A significant increase (p<0.05) in CP content to 9.84% was observed in RS prepared with high level of LW in comparison with 9.23% in RS samples prepared without LW. Similar result was obtained by Fallah (2019) in forage corn, according to the author; the increased LA production and low pH may contribute in reducing protein degradation. In a current study, the higher number of LAB in high LW level, and the significant increase in CP content which limited to RS samples prepared with that level of LW may confirms the LAB role in inhibition proteolysis during silage fermentation. The decrease in NH_3-N concentration in RS prepared with LW (Table 3) may provide evidence for the validity of that interpretation. Xu *et al.* (2022) reported that LAB improved the fermentation quality of silage and reduced protein degradation in silo.

The results of this study revealed that addition of LW regardless to its level increased (p<0.01) EE content of RS from 3.05 in RS samples prepared without LW to 3.39 and 3.51% in those prepared with low and high LW levels, respectively. Castaño and Villa (2017) pointed out that adding whey to Cuban grass at ensiling led to an increase in the EE content. Crude fiber content was decreased (p<0.01) from 45.88 in samples of RS prepared without LW to 43.79 and 42.23% in RS samples prepared with low and high LW levels, respectively.

The decrease in CF content of RS observed in a current study may be associated with rate of fermentation during ensiling which enhanced by LAB present in LW as evidenced by low pH and high LA concentration (Table 3). Rezaei *et al.* (2009) reported that ensiling leads to a reduction in CF content. Yanti and Yayota (2019) demonstrated that the addition of LAB during ensiling reduced the CF content through microbial degradation of carbohydrates released from the carbohydrates synthesized during silage.

Table 3: Effect of liquid whey and molasses levels on fermentation of reed plant silages (mean ± SE).

Items	liquid whey (LW), cfu/g FM			Molasses (M), %			P	
	0	Low	High	4	6	8	W	M
pH	5.03[a] ± 0.20	4.73[b] ± 0.07	4.45[c] ± 0.04	5.11[a] ± 0.16	4.74[b] ± 0.10	4.36[c] ± 0.02	**	**
LA, % of DM	2.80[c] ± 0.15	3.42[b] ± 0.18	4.79[a] ± 0.26	2.80[c] ± 0.19	3.74[b] ± 0.19	4.47[a] ± 0.31	**	**
VFA, % of DM	2.97[c] ± 0.11	3.37[b] ± 0.11	3.73[a] ± 0.15	2.99[c] ± 0.07	3.27[b] ± 0.13	3.81[b] ± 0.14	**	*
WSC, % of DM	4.71[a] ± 0.34	3.68[b] ± 0.26	2.62[c] ± 0.12	4.49[a] ± 0.34	3.82[b] ± 0.33	2.69[c] ± 0.12	**	**
NH3-N % of TN	6.21[a] ± 0.26	3.61[b] ± 0.14	2.91[c] ± 0.15	4.82[a] ± 0.21	4.10[b] ± 0.34	3.81[b] ± 0.31	**	**

Means with different superscripts in a row differ significantly at: * ($p<0.05$), ** ($p<0.01$); CFU: colony forming units; FM: fresh matter; LW: liquid whey; M: molasses; DM: dry matter; TN: total nitrogen; LA: lactic acid; VFA: volatile fatty acid; WSC: water soluble carbohydrate; NH_3-N: ammonia nitrogen.

The results showed a significant ($p<0.01$) increase in the NFE content in RS samples due to addition of LW. The increase in NFE content may be attributed to the high percentage of lactose naturally present in the whey. Castaño and Villa (2017) observed a significant increase ($p<0.05$) in NFE content in Cuba grass ensiled with whey at 50 ml/kg. Cajarville *et al.* (2012) reported that adding whey and molasses to silage leads to an increase in the content of the NFE content because they contain sugars.

With regard to the effect of adding different molasses levels, result revealed that DM content was increased ($p<0.05$) in samples of RS prepared with 8% level of M as compared with those prepared with other M levels 4 and 6%. This improvement may be attributed to the favorable fermentation process when soluble sugars are added to the desired microorganisms, which convert sugars to LA, lower pH, and inhibit aerobic activity. Mahala and Khalifa (2007) indicated that the addition of molasses increased DM content in sorghum silage. Desta *et al.* (2016) attributed the high DM content in silage to the high DM content of the added molasses. In a current study DM content of molasses was about 68% (Table 1).

The results showed a significant increase ($p<0.01$) in OM content with each increase in molasses level. The mean values were 79.20, 79.72 and 80.80% for RS samples prepared with 4, 6 and 8% molasses levels, respectively. Similarly, Khan *et al.* (2006) reported a gradual significant increase in OM content in grass silage with increasing levels of molasses. However, no significant changes were observed in OM content of date palm leaves ensiled with different levels of molasses (Saeed *et al.*, 2021). The possible early drop in pH due to the addition of increased levels of fermentable carbohydrates may have reduced nutrient losses during silage fermentation, resulting in increased OM contents. Yunus *et al.* (2000) pointed out to similar conclusion.

The results showed that addition of molasses at higher levels (6 and 8%) significantly ($p<0.01$) increased CP content of RS to 9.66 and 9.98%, respectively, as compared with addition of lower level, in which CP content was 8.83%. Farhan and Saeed (2022) obtained similar result. The increase in CP content can be explained on basis of proteolytic activity which may be inhibited by a rapid decrease in pH associated with an increase in fermentable sugars. The increase in CP content was attributed to reduce proteolytic process during fermentation and decease ammonia-N (NH_3-N) concentration (Bai *et al.*, 2024). The significant decrease in NH_3-N concentration has been detected in RS samples prepared with higher levels of M as compared with lower level (Table 3).

The EE content was increased ($p<0.01$) from 2.99% in RS samples prepared with 4% M to 3.48 and 3.48% in those prepared with 6 and 8% levels of M, respectively. This increase may be due to the role of the added molasses, which improved the fermentation pattern by supplying the silage microorganisms with more soluble sugars to produce organic acids including volatile fatty acids (VFA). Saeed and Muhamad (2017) reported that providing silage microbes with increased amounts of fermentation substrate (WSC) increased production of VFA. Similar to the result observed in a current study, a trend of increasing EE content in corn stover associated with addition of increased levels of molasses was obtained by Farhan and Saeed (2022).

The results revealed that CF content in RS was significantly ($p<0.01$) decreased with increasing levels of molasses added at ensiling. The mean values were 45.13, 43.95, and 42.82% for RS samples prepared with 4, 6 and 8% molasses, respectively. Similar result was obtained in DPL samples ensiled with different levels of molasses (Saeed *et al.*, 2021). The positive effect of molasses on CF content was reported by Bautista-Trujillo *et al.* (2009) and Bureenok *et al.* (2012) in different silages. The decrease in CF content may be due to degradation of cell wall component in RS silages as a response to enhanced silage fermentation caused by providing silage microorganism with more of fermentable sugar (Bautista-Trujillo *et al.*, 2009). Role of increased M levels added at ensiling on fermentation quality was shown (Table 3). Another possible reason for the lower CF content may be related to the dilution effect of low fiber content in molasses (Table 1). Similar explanation was suggested by Castaño and Villa (2017) in Cuba grass silage.

Content of NFE was significantly ($p<0.01$) increased as affected by increasing M levels added at ensiling, however, the increase in that nutrient was limited to higher level of M as compared with other levels. The mean values were 22.24, 22.63, and 24.51% for the RS samples prepared with 4, 6 and 8% molasses, respectively. This increase may be attributed to the role of molasses as a rich source of fermentable sugars, enhancing the activity of anaerobic bacteria responsible for the fermentation process. However, no significant changes in NFE content were observed due to ensiling DPL with different levels of molasses (Saeed *et al*., 2021).

Regarding the effect of the interaction between LW and M levels on the chemical composition of reed plant silage, statistical analysis (Table 4) revealed that all nutrients were significantly affected by this interaction. Higher DM content was ($p<0.05$) observed in RS samples prepared with adding LW, especially when M was added at higher levels, compared to DM content in 0LW6M and 0LW8M. This result may be attributed to the effect of accumulation of some elements in additives, whey and molasses. Kanengoni *et al*. (2016) reported that fresh whey contains WSC, soluble protein; molasses also contains significant amounts of WSC. The abundance of sugars in silage leads to increased production of lactic acid, which limits the decomposition of DM during fermentation.

The results revealed higher contents ($p<0.05$) of OM was found in HLW6M, HLW8M and LLW8M compared to other samples, with the highest values of 81.81% associated with the samples prepared with high levels of both LW and M. This improvement is may be attributed to the role of LAB when the high level of whey is added along with the molasses level, which provides the silage with large amounts of soluble sugars to produce large amounts of LA, leading to reduce the pH.

The results showed that higher CP content was associated with LW and higher M levels treated RS samples. This was expected since whey contained soluble proteins, which contribute to the protein content of the silage samples, in addition to the role of LAB, which produce large amounts of LA to lower the pH, this reduces protein degradation by inhibiting the activity of aerobic organisms. The lower CP content was found in 0LW4M and LLW4M. Kadi *et al*. (2018) explained such decrease in CP content by the fact that proteins may be bound to cell walls, as is typically found in roughage.

The results revealed that lower EE content was found in 0LW4M, 0LW6M and LLW4M compared with other RS samples. The higher EE content associated with LW treated RS samples may be attributed to the role of the added whey, which contains a percentage of residual milk fat, in addition to the improved fermentation process and the lower pH, which leads to inhibition of aerobic activity and consequently an increase in the EE content. As for the effect of molasses, increasing its level in the silage increases the concentration of soluble sugars, which leads to an increase in the concentration of the EE through the production of VFA from the oxidation of WSC during anaerobic fermentation. Saeed and Mohammed (2017) reported that providing silage microbes with increasing amounts of WSC led to an increase in the concentration of VFA.

Table 4: Effect of interaction between levels of liquid whey and molasses on chemical composition of reed plant silages (mean ± SE).

Liquid whey, LW	**0**			**low**			**high**			**P**
Molasses, M, %	**4**	**6**	**8**	**4**	**6**	**8**	**4**	**6**	**8**	
DM	28.56^{d} ± 0.39	28.53^{d} ± 0.25	28.98^{cd} ± 0.43	29.36^{bcd} ± 0.50	29.19^{bcd} ± 0.22	30.73^{a} ± 0.27	29.92^{abc} ± 0.29	30.17^{ab} ± 0.24	29.94^{abc} ± 0.24	*
OM	79.68^{c} ± 0.22	78.58^{d} ± 0.32	79.89^{c} ± 0.19	78.24^{d} ± 0.11	79.40^{c} ± 0.23	80.71^{b} ± 0.25	79.67^{c} ± 0.24	81.20^{ab} ± 0.18	81.81^{a} ± 0.21	*
CP	8.60^{d} ± 0.17	9.46^{bcd} ± 0.20	9.65^{abc} ± 0.19	8.75^{cd} ± 0.17	9.64^{abc} ± 0.24	9.81^{ab} ± 0.25	9.16^{bcd} ± 0.22	9.88^{ab} ± 0.18	10.49^{a} ± 0.26	*
EE	2.61^{c} ± 0.10	3.13^{b} ± 0.11	3.41^{ab} ± 0.07	3.08^{b} ± 0.09	3.66^{a} ± 0.09	3.43^{ab} ± 0.09	3.30^{ab} ± 0.12	3.64^{a} ± 0.13	3.59^{a} ± 0.14	*
CF	47.33^{a} ± 0.54	45.97^{ab} ± 0.77	44.35^{bcd} ± 0.76	44.80^{bc} ± 0.68	43.63^{cd} ± 0.93	42.94^{cde} ± 0.61	43.27^{cde} ± 1.02	42.24^{de} ± 0.54	41.18^{e} ± 0.24	*
NFE	21.14^{e} ± 0.25	20.01^{e} ± 0.64	22.47^{cde} ± 0.67	21.61^{de} ± 0.68	22.46^{cde} ± 0.55	24.53^{abc} ± 0.53	23.94^{bcd} ± 0.56	25.43^{ab} ± 0.67	26.54^{a} ± 0.44	*

Means with different letters at the same row are significantly different at: * ($p<0.05$), ** ($p<0.01$). LW: liquid whey; M: molasses; DM: dry matter; OM: Organic matter; CP: crude protein; EE: ether extract; CF: crude fiber; NFE: nitrogen free extract.

The lower CF content shown in LW treatments especially with higher M levels may be due to the improved fermentation processes as affected by the addition of LW rich in LAB, which contributed to LA production and lowered pH. This decrease in CF content may have resulted from increased cell wall decomposition due to improved silage fermentation caused by these additives (Bautista-Trujillo *et al*., 2009).

The results showed that higher NFE content were found in LW treated RS samples and higher M levels. This increase may be due to the enhanced fermentation process resulting from the addition of LW. In addition, to the important role of molasses in enhancing fermentation process by providing LAB with fermentable sugars.

Silage fermentation characteristics

Table 3 shows the fermentation characteristics of reed plant silage (RS) as affected by adding different levels of liquid whey (LW) and molasses (M). Results revealed that adding low and high levels of LW intended to introduce LAB at 1×10^5 and 1×10^6 cfu/g FM led to a significant ($p<0.01$) decrease in the pH value to 4.73 and 4.30 respectively as compared with 5.03 in RS samples prepared without LW. Gültekin and Kaya (2022) indicated that adding whey to grass reduce pH, and improve silage quality. Similar pH value of 4.3 was reported by Al-Sultani (2016) in reed plant ensiled with addition of urea at level of 1% and 10% of date juice.

The decrease in pH in LW treated RS samples can be explained by the ability of LAB to ferment soluble sugars into LA during fermentation. In conjunction with that result, a gradual significant increase in LA concentration was associated with the level of LW. Beyzi *et al*. (2023) reported that LAB proliferate in the silo, thus producing organic acids that are very effective in lowering pH and inhibit the development of undesirable microorganisms.

Higher ($p<0.01$) LA concentration was detected in RS samples prepared with addition of LW. Mean LA concentration were increased from 2.80 in RS samples ensiled without LW to 3.42 and 4.79% DM in those ensiled with addition of low and high levels of LW, respectively. This increase in acid production may be attributed to supply ensiled materials with increased number of LAB through the increased level of LW added at ensiling. These bacteria, in turn, produce large quantities of LA, thereby improving the fermentation process. Ohshima *et al*. (1997) emphasized that adding whey can double the number of LAB, stimulate LA production, and improve the quality of silage fermentation. Nkosi and Groenwald (2012) observed that the addition of whey increased ($p<0.05$) LA concentrations. This is consistent with finding of Bautista-Trujillo *et al*. (2009) in which, they noticed the positive effect of ensiling maize grass with addition of whey on LA content. In line with result obtained in a current study, a significant increase in LA production in corn plant silage was found due to addition of whey and molasses (Fallah, 2019).

Result showed an increase ($p<0.01$) in the TVFA concentrations with addition of LW and increased its level. The mean values were 2.97, 3.37 and 3.73% of DM in samples of RS prepared with low and high levels of LW compared with 2.97 % of DM in RS samples prepared without LW. The increase in TVFA concentration may be due to improved silage fermentation by increasing the number of LAB. Schumcher *et al*. (2019) reported that whey is a product that may be able to favor the production of organic acids and LA in anaerobic environment.

Results also revealed that the residual WSC content was decreased ($p<0.01$) from 4.71 in RS samples prepared without LW to 3.68 and 2.62% of DM in those treated with low and levels of LW, respectively. This decrease is due to the increased consumption of soluble sugars by LAB producing LA during anaerobic fermentation as evidenced in a noticeable increase in LA content together with decrease in pH. Fabiszewska *et al*. (2019) reported that lower residual WSC may be caused by a greater utilization of WSC to produce a high amount of LA. Similarly, the decrease ($p<0.01$) in WSC content was observed in temperate forage ensiled with whey (Cajarville *et al*., 2012). Nkosi and Groenwald (2012) considered the lower ($p<0.05$) residuals of WSC after 40 days of ensiling in whey treated silage an indication that more sugar was utilized by LAB to produce LA.

In consistence with the effect of adding LW on fermentation characteristics previously mentioned, result revealed that NH_3-N concentration was decreased ($p<0.01$) from 6.21 % of TN in RS samples prepared without addition of LW to 3.61 and 2.91% of TN in those prepared with low and high levels of LW, respectively. The reason for these decreases may be associated with the presence of LAB, which leads to a decrease in pH and prevents the growth of harmful microorganisms responsible for protein degradation during silage. Zanin *et al*. (2022) indicated that a rapid decrease in pH of silage maintains protein content, reducing NH_3-N concentration. The decrease in NH_3-N with whey and LAB inoculation was confirmed by many other workers (Nkosi and Groenewals, 2012; Bautista-Trujillo *et al*., 2009).

Regarding effect of adding different levels of M on silage fermentation, results showed that there was a significant decrease ($p<0.01$) in pH values with each increase in the level of M., mean values were, 5.11, 4.74 and 4.36 for the samples prepared with 4, 6, and 8% of M, respectively. The decrease in pH can be related to the increase in the WSC content of the ensiled materials. Considering that such additional WSC would stimulate fermentation leading to reduce pH (Bai *et al*., 2024). Cajarville *et al*. (2012) reported that ensiling temperate forages with molasses increased their WSC content causing noticeable decrease in the pH.

The results revealed that increasing levels of molasses added at ensiling reed plant led to a significant increase ($p<0.01$) in the LA concentration. The mean values were 2.80, 3.74, and 4.47% of DM for 4, 6 and 8% M levels, respectively. This increase can be attributed to the activity of LAB converting molasses added as a source of WSC to LA as the final product of the metabolism of these bacteria. Beyzi *et al*. (2023) observed that the presence of sufficient sugars leads to an increase in the number of LAB in the silo and is very effective in producing LA and lowering the pH of silage mass.

The significant increase detected in LA concentration in the current study was accompanied with significant increase ($p<0.05$) in VFA concentration, however, the significant increase was not associated with every increase in M levels as was with LA concentration. The mean values of VFA were 2.99, 3.27, and 3.81% of DM for RS prepared with M level of 4, 6 and 8% respectively. This increase is a natural process occurred at anaerobic condition, by which organic acids are produced as a result of silage microorganism's metabolism of available soluble sugars. Schroeder (2013) reported that VFA represent the final product of the decomposition or metabolism of soluble sugars in anaerobic conditions of silage. Similar to the result obtained in a current study, concentration of VFA adopted an increase ($p<0.01$) with increasing level of date honey as a source of WSC (Saeed and Mohammad, 2017).

Inconsistence with increasing LA and VFA concentrations, results showed that the residual WSC content was significantly decreased ($p<0.01$) with increasing levels of M added at ensiling of reed plant. The mean values were 4.49%, 3.82%, and 2.69% of DM for samples of RS prepared M at levels of 4, 6, and 8%, respectively. This decrease is due to increased consumption of soluble sugars by LAB and their assimilation to ensure increased activity during ensiling. Rehman (2011) demonstrated that soluble sugars are the primary substrate for LAB. Therefore, the presence of sufficient WSC will ensure that the LAB need to dominate silage fermentation has been secured. Nkosi *et al*. (2009) confirmed an increase in WSC content upon addition of molasses during storage in silage.

The effect of increasing M levels on the concentration of NH_3-N adopted descending trend in a current study. There were decreases ($p<0.01$) associated with each increase in the level from 4 to 6 and 8%, the corresponded values were 4.82 to 4.10 and 3.81% of TN, respectively. This decrease in the NH_3-N concentration may be due to the inhibition of proteolytic activity. A rapid decrease in the pH may also participate in the decrease in NH_3-N content by inhibition of aerobic microorganisms and enzymes responsible for protein degradation during silage fermentation (Martinez-Fernandez *et al*., 2013).

Regarding the effect of the interaction between LW and M levels on the fermentation characteristics of reed plant silage, the result (Table 5) showed that all fermentation characteristics were significantly affected by this interaction. The lower pH values were clearly associated with LW treatment especially at higher M levels. Such response was somewhat expected due to the certain presence of LAB in the fermentation medium. In line with that interpretation,

Table 5: Interaction effect between liquid whey and molasses levels on fermentation of reed plant silages (mean ± SE).

Liquid whey, LW	0			Low			High			P
Molasses, M, %	4	6	8	4	6	8	4	6	8	
pH	5.87^{a} ± 0.19	4.84^{bc} ± 0.13	4.38^{de} ± 0.14	4.97^{b} ± 0.10	4.80^{bcd} ± 0.09	4.41^{cde} ± 0.10	4.48^{cde} ± 0.07	4.57^{bcde} ± 0.05	4.31^{e} ± 0.04	**
LA, % of DM	2.14^{f} ± 0.19	2.93^{e} ± 0.08	3.32^{d} ± 0.12	2.51^{f} ± 0.11	3.70^{c} ± 0.05	4.06^{c} ± 0.12	3.74^{c} ± 0.07	4.59^{b} ± 0.18	6.03^{a} ± 0.15	**
VFA, % of DM	2.74^{d} ± 0.10	2.88^{cd} ± 0.16	3.28^{c} ± 0.25	3.08^{cd} ± 0.07	3.16^{cd} ± 0.16	3.86^{ab} ± 0.10	3.14^{cd} ± 0.15	3.76^{b} ± 0.19	4.30^{a} ± 0.12	*
WSC, % of DM	5.82^{a} ± 0.34	5.14^{ab} ± 0.45	3.17^{cd} ± 0.10	4.61^{b} ± 0.38	3.80^{c} ± 0.25	2.63^{de} ± 0.16	3.05^{cde} ± 0.09	2.52^{de} ± 0.22	2.28^{e} ± 0.13	**
NH_3-N % of TN	7.41^{a} ± 0.30	5.82^{b} ± 0.28	5.41^{b} ± 0.13	4.06^{c} ± 0.21	3.55^{cd} ± 0.10	3.21^{cd} ± 0.25	2.98^{de} ± 0.11	2.94^{e} ± 0.08	2.81^{e} ± 0.07	**

Means with different letters at the same row are significantly different at: * ($p<0.05$), ** ($p<0.01$). CFU: colony forming units; FM: fresh matter; W: liquid whey; M: molasses; DM: dry matter; TN: total nitrogen. LA: lactic acid; VFA: volatile fatty acid; WSC: water soluble carbohydrate; NH_3-N: ammonia nitrogen.

Links Researchers

Bautista-Trujillo *et al*. (2009) pointed out that molasses contains large quantity of WSC, whereas, liquid whey contains WSC, protein and some LAB, which could benefit the fermentation process. Results obtained by Castaño and Villa (2017) indicated better fermentation in tropical grass ensiled with the mixture of molasses and whey. According to that finding, the highest ($p<0.01$) pH value in 0LW4M can be understood.

Higher ($p<0.01$) LA concentrations were detected in the RS samples prepared with LW in combination with 6 and 8% M level, with the highest value (6.03% of DM) in HLW8M. This may be due to the LAB being supplied with large amounts of soluble sugars to produce large amounts of LA, which in turn is responsible for lowering the pH. The lowest concentrations were detected in 0LW4M and 0LW6M. However, 0LW8M recorded higher ($p<0.01$) LA concentration than other RS samples that prepared without LW. The response to high M level in case no LW was added may be due to epiphytic LAB benefiting from the influx of substantial amount of soluble sugar.

Statistical analysis revealed that VFA concentration was affected by the level of molasses more than that of liquid whey. Higher ($p<0.05$) value was detected in LLW8M (3.86% of DM). At high level of LW, high VFA ($p<0.05$) values were detected in both HLW6M and HLW8M (3.76 and 4.30% of DM). The lowest concentrations of these organic acids were recorded in other RS samples. Saeed (2017) reported that soluble sugar is rapidly decomposed by LAB lactic acid bacteria to produce lactic acid and other organic acid, thus reduce the pH of silage.

The changes previously mentioned in fermentation characteristics of RS in the current study are expected to be reflected on the residual WSC content. As shown in Table 5, the lowest concentration of residual WSC was associated with well fermented RS samples.

As evidenced by low pH and higher LA concentration, RS prepared with both additives, LW and M showed well fermented characteristics. Therefore, lower WSC content was expected because such desirable fermentation changes are obtained through sugar fermentation (Bilal, 2009). These two additives provide WSC to be fermented by LAB present in LW for lactic acid fermentation (Bautista-Trujillo *et al*., 2009). Accordingly, lower ($p<0.01$) residual WSC was detected in LLW6M, LLW8M, HLW6M and HLW8M.

Concentration of NH_3-N was significantly affected by the interaction between LW and M levels. The lowest ($p<0.01$) values were detected in RS samples prepared with HLW followed by those prepared with LLW. The reason behind these decreases especially in HLW8M may be attributed to the decrease in protein degradation processes resulting from Clostridium bacteria and the inhibition of their activity through the acidic environment dominated by LAB through increased LA production, which in turn lowers the pH and inhibits harmful microorganisms.

Amanullah *et al*. (2014) demonstrated that the higher NH_3-N content in barley forage ensiled without inoculation with LAB. The RS samples prepared without addition of LW had the highest NH_3-N concentration regardless to M levels.

CONCLUSION

In conclusion it was shown that reed plants can be effectively ensiled with liquid whey and molasses as indicated by desirable changes in chemical composition, especially an increase in DM, CP and EE contents together with a decrease in CF contents. Reed plant ensiled with those additives was efficiently fermented as evidenced by low pH and NH_3-N accompanied with high lactic acid production and volatile fatty acids. Moreover, as a natural source of LAB, it is beneficial to add liquid whey to water used to adjust the DM level of reed plant and to dilute the molasses required for silage fermentation.

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NOVELTY STATEMENT

It is the first study on silage in animal nutrition.

AUTHOR'S CONTRIBUTION

Qasim performed the laboratory and field work and Ali did the final preparation of the manuscript.

Generative AI and AI-assisted technology statement

We confirm that artificial intelligence was not used in preparing the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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