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Effect of fermented juice of lactic acid bacteria on structural carbohydrates and nutritional value of wheat straw silage

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ABSTRACT

The current study aimed to investigate the effect of ensiling wheat straw (WS) with addition of fermented juice of lactic acid bacteria (FJLB) prepared from wild reed plant with different sources and levels of soluble carbohydrates on changes in structural carbohydrates content and nutritive value of straw. The FJLBs were prepared by incubating wild reed plants with cane or date molasses at three levels, 0, 3, and 5% for each, and were added to WS at level of 1%, together with urea and molasses at a level of 1 and 10% respectively. The silage samples were packed in double plastic bags and kept anaerobically for 45 days. The results showed a significant decrease in the content of neutral detergent fiber (NDF, $P<0.05$), acid detergent fiber (ADF) and cellulose ($P<0.01$) in WS silage with increasing the level of soluble carbohydrates added at preparation of the FJLB. Ensiling WS with addition of FJLB prepared with a high level of those soluble carbohydrates resulted in a significant increase ($P<0.01$) in the in vitro digestibility of dry matter (IVDMD) and expected intake of dry matter (DMI), as well as a significant improvement ($P<0.05$) in the relative feed value (RFV) of the WS. The results of the current study also reached the importance of the interaction between the source and level of soluble carbohydrates used in preparing the FJLB in affecting the components of the cell wall and some characteristics of the nutritional value of wheat straw silage, The results showed a significant decrease ($P<0.05$) in the content of acid detergent fiber (ADF) and cellulose, as well as an increase ($P<0.05$) in the in vitro digestibility of dry matter (IVDMD)and relative feed value (RFV) of the WS.

تأثير العصير المتخمر لبكتيريا حامض اللاكتيك على الكربوهيدرات التركيبية والقيمة الغذائية لسايلج تبن الحنطة

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الخلاصة

هدفت الدراسة الحالية التحري عن تأثير سيلجة تبن الحنطة بإضافة العصير المتخمر لبكتيريا حامض اللاكتيك المحضر من نبات القصب البري مع مصادر ومستويات مختلفة من الكربوهيدرات الذائبة على التغير في محتوى التبن من الكربوهيدرات التركيبية وبعض معايير قيمته الغذائية. حضر العصير بحضن نبات القصب البري مع مولاس القصب او التمر بثلاث مستويات, 0 و 3 و 5% لكل منهما واضيف عند سيلجة التبن بمستوى 1%, سوية مع اليوريا والمولاس بمستوى 1 و 10% على التوالي. عبئت نماذج السايلج في أكياس بلاستيكية مزدوجة وحفظت لاهوائيًا لمدة 45 يوما. اظهرت النتائج حصول انخفاض معنوي في المحتوى من مستخلص الالياف المتعادل (P<0.05) ومستخلص الالياف الحامضي والسليلوز (P<0.01) في نماذج سايلج تبن الحنطة بزيادة مستوى الكربوهيدرات الذائبة المضافة عند تحضير العصير المتخمر لبكتيريا حامض اللاكتيك. وادت سيلجة تبن الحنطة بإضافة العصير المتخمر المحضر مع المستوى المرتفع من تلك الكربوهيدرات الذائبة الى حصول زيادة معنوية (P<0.01) في الهضم المختبري للمادة الجافة والتناول المتوقع من المادة الجافة, فضلا عن حصول تحسن معنوي (P<0.05) في القيمة الغذائية النسبية للتبن. كذلك توصلت نتائج الدراسة الحالية الى اهمية التفاعل بين مصدر و مستوى الكربوهيدرات الذائبة المستخدمة في تحضير العصير المتخمر في التأثير على مكونات جدار الخلية و بعض خصائص القيمة الغذائية لسايلج تبن الحنطة, اذ اظهرت النتائج حصول انخفاض معنوي (P<0.05) في محتوى مستخلص الالياف الحامضي و السليلوز فضلا عن زيادة (P<0.05) الهضم المختبري و القيمة الغذائية النسبية للتبن .

الكلمات الافتتاحية: تبن الحنطة, السيلجة, العصير المتخمر لبكتيريا حامض اللاكتيك, القصب البري

INTRODUCTION

Roughages are considered the main source of ruminants feed, and it is expected that these animals will continue depending on these feeds due to the expansion of human activities and competition on ingredients of concentrates. Straws, especially wheat straw are considered the most available roughages for breeders, despite its low nutritive value due to low content of protein and vitamins and the high content of cell walls and lignin (Saeed, 2012). Where, these components including cellulose and hemicellulose are linked to lignin (Lee, *et. al*., 2014). However, such links can be broken by means of suitable treatments, making cellulose and hemicellulose more available to rumen microbes.

Silage is a common practice in many countries to preserve excess crops and ensure the availability of feed throughout the year. Kung, *et. al*. (2003) reported that ensiling is a complex biochemical process that depends on the fermentation of soluble carbohydrates by lactic acid bacteria (LAB) under anaerobic condition. Saeed (2015) pointed out the possibility of producing more palatable feed from crop residues by ensiling.

Producing good quality silage requires ensuring the activity of LAB by metabolizing soluble carbohydrates as a substrate to produce sufficient amounts of lactic acid (LA) to lower the pH (McDonald et al., 1991). This indicates the importance of using appropriate additives to improve the quality of fermentation.

The FJLB is considered one of the natural additives, it is easily prepared at a low cost by culturing epiphytic LAB attached to crops and their waste before ensiling, and taking advantage of the cultured LAB to start silage fermentation (Masuko, *et. al*., 2002). Bureenok *et. al*. (2005) demonstrated that preparing the FJLB by anaerobic incubation the ensiled crop with water for two days would enhance the numbers and activity of LAB naturally present on crops to be used as an additive to silage.

To our knowledge, FJLB has not been prepared neither from reed plant nor with addition of date molasses as soluble sugar to be used as an additive at ensiling straw. Therefore, the current study aimed to investigate the effect of ensiling wheat straw with FJLB prepared form wild reed plant with different levels of cane or date molasses on cell wall components and nutritive value of straw.

MATERIAL AND METHOD

The FJLB was prepared from wild reed plants based on cane or date molasses as sources of soluble carbohydrates at three levels (w/v): 0, 3, and 5% for each, according to the method mentioned by Masuko et al. (2002) and Bureenok et al. (2016). Where, 200 g of fresh cut wild reed plant was mixed with 1000 ml of sterilized distilled water in a blender for 5 minutes. The mixture was then filtered through a double layers of cheese cloth and distributed into 6 glass bottles, to which a source of soluble carbohydrates used in the study was added at the levels indicated above. The bottles were covered tightly and incubated anaerobically at 30°C for 48 hours.

Six samples of ground wheat straw silages were made by adding the FJLB at a rate of 1% (v/w), urea as a source of nitrogen at a rate of 1%, and molasses as a source of soluble sugars at a rate of 10% as silage additives. Urea and molasses were dissolved in an appropriate amount of water to ensure a level of dry matter (DM) in the mixture before ensiling of about 35%. Table 1 shows some characteristics of the chemical composition and nutritive value of wheat straw before ensiling.

Table 1: Some characteristics of chemical composition and nutritive value of wheat straw

Item	%
Dry matter , DM	94.20
Crud protein, CP	1.47
Ether extract, EE	1.42
In vitro dry matter digestibility, IVDMD	41.81
The expected dry matter intake, DMI (% BW)	1.61
The relative feed value, RFV	52.18
Neutral detergent fiber, NDF	74.64
Acid detergent fiber, ADF	47.61
Acid detergent lignin, ADL	17.19
Cellulose	30.42
Hemicellulose	27.03

The silage materials were packed in double plastic bags with a capacity of 2 kg, 4 replicates for each sample, and they were well compressed to expel the air from them and closed tightly. These samples were transferred to a suitable storage place free of rodents and insects after placing replicates of each sample in a dark bag. The samples were stored separately and covered tightly until the end of the silage period, which was specified as 45 days. After the end of the storage period, the silage samples were opened and samples were taken to perform the planned analyses.

Chemical composition of wheat straw and silages including DM, CP and EE were determined according to AOAC (2005). Cell wall components including NDF, ADF, ADL, cellulose and hemicellulose in both straw and silages were determined according to the method described by Goering and Van Soest (1970).

In vitro dry matter digestibility (IVDMD) in samples of straw and silages were determined according to the method of Tilley and Terry (1963). Where, about of 1 g of dried straw and silage samples were incubated with rumen liquid from the rumen of a sheep obtained from the slaughterhouse. Ten ml of strained rumen liquid together with 40 ml of artificial saliva were placed in glass tubes and placed in a water bath with temperature previously regulated at 37°C. The incubation continued for 48 hours with CO_2 gas pumped twice during the day. The IVDMD was estimated according to the following equation:

$$\textbf{IVDMD \%} = \frac{\textbf{(DM sample} - \textbf{(DM residue sample} - \textbf{DM residue blank))}}{\textbf{DM sample}} \times \textbf{100}$$

The expected dry matter intake (DMI was estimated according to the following equation derived by NRC (2001):

DMI (% of BW) =120 / NDF

The relative feed value (RFV) was estimated according to the following equation derived by Linn, *et. al*. (1987)

RFV, % = (DMD × DMI) / 1.29

The data were analyzed statistically according to factorial experiments (2×3) using a completely randomized design CRD. The SAS (2010) statistical analysis program was used to analyze the data statistically and Duncan's (1955) multiple borders test was used to compare the means.

RESULTS AND DISCUSSION

Table 2 shows the effect of ensiling wheat straw with addition of FJLB prepared with different sources and levels of soluble carbohydrates on cell wall composition. The results revealed that there was no significant effect of the source of soluble carbohydrates used in preparation of FJLB on the content of NDF, ADF, ADL, cellulose and hemicellulose. While the level of those sources had a significant effect on these cell wall components. Higher NDF (66.47%, P<0.05) and ADF (45.38%, P<0.01) contents were associated with WS samples ensiled with FJLB prepared without soluble carbohydrates. The lowest NDF content (P<0.05) was observed in WS samples ensiled with the addition of FJLB prepared with soluble carbohydrates at level of 5%, though it was not significantly differed as compared with those ensiled with soluble carbohydrates at level of 3%. With regard to ADF, it was significantly decrease (P<0.01) as level of soluble carbohydrates used in preparation of FJLB was increased.

Table 2- Shows effect of ensiling wheat straw with different sources and levels of FJLB on cell wall components (mean ± SE)

Item	Source of FJLB		Levels, %			*P*	
	Cane-M	Date-M	0	3	5	S	L
NDF	64.81 0.74±	65.09 0.58±	66.47^{a} 0.56±	64.53^{ab} 0.83±	63.86^{b} 0.75±	NS	*
ADF	43.01 0.64±	43.67 0.51±	45.38^{a} 0.24±	43.19^{b} 0.52±	41.46^{c} 0.46±	NS	**
ADL	14.29 0.29±	14.45 0.31±	14.60 0.29±	14.23 0.48±	14.29 0.32±	NS	NS
Cellulose	28.72 0.71±	29.22 0.57±	30.78^{a} 0.43±	28.96^{ab} 0.88±	27.17^{b} 0.34±	NS	**
Hemicellulose	21.80 0.54±	21.42 0.40±	21.09 0.24±	21.34 0.50±	22.40 0.61±	NS	NS

NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin. Means with horizontally different letters are significantly differed at * (P<0.05); ** (P<0.01); NS = non-significant

Similar results were obtained by Yahaya et al. (2004) and Bureenok et al. (2012) who showed that the content of NDF and ADF in silage was constantly reduced with the addition of FJLB in silage compared to control group, indicating an improvement in the nutritive value of silage. Yanti and Yayota (2019) found that the lowest NDF content was observed in the agricultural by-product mixture ensiled with FJLB. In contrast, Lukkananukool, et al. (2018) noticed that higher (P<0.05) NDF content was observed in the cassava samples ensiled with addition of FJLB prepared with soluble carbohydrates, while the ADF content did not differ. Jin-ling et al. (2013) reported that there were no significant differences in NDF and ADF contents in samples of rice straw ensiled with FJLB prepared with soluble sugars.

The NDF and ADF contents in a current study were decreased in the samples of WS ensiled with FJLB prepared without addition of soluble carbohydrates compared to the wheat straw before ensiling (Table 1). The NDF content in the silage was decreased from 74.64 to 66.47%, but the ADF content was slightly decreased from 47.61 to 45.38%. The variable decreases in those structural carbohydrates, reveals that hemicellulose is more susceptible to decomposition during ensiling compare to cellulose. Such the beneficial effects towards the silage may lead to an increase in the digestibility of silage (Yahaya et al., 2004 and Pitiwittayakul et al., 2021). Huiseden et al. (2009) reported that the NDF content decreased after long fermentations, which may be due to enzymatic or acidic hydrolysis of the cell wall fraction. Low fiber content using LAB or FJLB has also been reported in some studies (Yahaya et al., 2004 and Denek et al., 2012).

Table 3 shows the effect of interaction between sources and levels of soluble carbohydrates used in preparation of FJLB added at ensiling wheat straw on changes in cell wall components. The results showed that NDF, ADL and hemicellulose contents in WS silages were not significantly affected, however, ADF and cellulose contents were significantly responded to that interaction. The lowest (P<0.05) ADF content were associated with the samples of WS ensiled with FJLB prepared with addition of cane molasses at higher level (40.76%), while the highest (P<0.05) contents were observed in the samples of WS ensiled with the addition of FJLB, prepared without soluble carbohydrates (45.38%).

Table 3: Changes in cell wall components as affected of interaction between sources and levels of soluble carbohydrates used in preparation of FJLB added ensiling of wheat straw (mean ± SE)

WSC	**Cane- M**			**Date-M**			***P***
Levels	**0**	**3**	**5**	**0**	**3**	**5**	
NDF	66.47 0.86±	64.13 1.26±	63.84 1.52±	66.47 0.86±	64.93 1.25±	63.88 0.57±	NS
ADF	45.38[a] 0.37±	42.91[b] 0.69±	40.76[c] 0.61±	45.38[a] 0.37±	43.48[b] 0.86±	42.17[bc] 0.56±	*
ADL	14.60 0.45±	14.06 0.74±	14.23 0.38±	14.60 0.45±	14. 41 0.71±	14.34 0.58±	NS
Cellulose	30.78[a] 0.66±	28.85[ab] 1.41±	26.53[b] 0.45±	30.78[a] 0.66±	29.07[ab] 1.27±	27.83[b] 0.26±	*
Hemicellulose	21.09 0.87±	21.22 0.59±	23.08 1.14±	21.09 0.87±	21.45 0.90±	21.71 0.36±	NS

NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin; Means with horizontally different letters are significantly differed at * ($P<0.05$); NS = non-significant

The decrease in ADF content in samples of WS ensiled with addition of FJLB may due to the improvement in the quality of fermentation (Harrison, *et. al*., 1994). McDonald, *et. al*. (1991) reported that increasing the number of LAB improve the quality of silage. Huisden, *et. al*. (2009) found that the addition of molasses and long-term fermentations reduced the ADF content. Baytok et al. (2005) concluded that lower fiber content in silage was a result of increased cell wall hydrolysis.

The effect of the mentioned interaction also revealed that ensiling WS with the addition of FJLB prepared from higher level of soluble carbohydrates regardless to the source, whether it was cane or date molasses, decreased ($P<0.05$) cellulose content to 26.53 and 27.82%, respectively, compared to the control treatment (30.78%). Abubakr et al. (2021) and Yanti and Yayota (2019) showed that the addition of FJLB prepared with soluble carbohydrates led to a reduction in plant cell wall components through microbial degradation of carbohydrates liberated from structural carbohydrates during ensiling.

McDonald et al. (1991) reported that structure carbohydrates are not substrate for fermentation of LAB in silage. However, studies conducted by Denek, *et. al*. (2012) indicated that ensiling alfalfa with FJLB contained a lower content of components of fibrous cell walls. It was suggested that the reduction in cell wall content due to addition of FJLB was a result of hydrolysis resulting from the action of some bacteria capable of breaking down cellulose and hemicellulose (Wanapat, *et. al*., 2013), or through enzymes present in FJLB (Yanti et al., 2019).

Table 4 shows the effect of ensiling wheat straw with addition of FJLB prepared with different sources and levels of soluble carbohydrates on some characteristics of nutritive value including in vitro dry matter digestibility (IVDMD), expected dry matter intake (DMI) and relative feed value (RFV). The results revealed that those characteristics were not significantly affected by the source of soluble carbohydrates used in preparation of FJLB. However, a significant (P<0.01) increase in IVDMD with increasing level of soluble carbohydrates was observed. The highest (46, 59%, P<0.01) value was recorded in samples of WS ensiled with the addition of FJLB prepared with soluble carbohydrates at level of 5%. Similar result was obtained by Cao, *et. al*. (2013).

The improvement in IVDMD in a current study may be a direct result of enhancing the activity of LAB present in the FJLB by increasing the level of soluble sugars and silage fermentation accordingly. Cai, *et. al* (2003) observed an increase in the IVDMD of rice straw silage as a result of the addition of LAB at ensiling, the researcher attributed this increase to the effect of inoculation in reducing the DM loss during silage fermentations.

Results also showed that the expected DMI was significantly affected by the level of soluble carbohydrates added in preparation of FJLB, with the highest value (P<0.01) of about 1.88% of BW recorded in samples of WS ensiled with the addition of FJLB prepared with a higher level 5% soluble carbohydrates. This increase may be due to the decrease in NDF and ADF contents in this silage (Table 2). Similar finding was obtained by Bureenok et al. (2012). Lukkananukool, *et. al*. (2018) found that ensiling cassava leaves and Napier grass with FJLB led to improve feed intake, digestibility and nutritive characteristics compared to the control.

Table 4: Shows effect of ensiling wheat straw with different sources and levels of FJLB on some characteristics of nutritive value (mean ± SE)

Item	Source of FJLB		Levels, %			*P*	
	Cane-M	Date-M	0	3	5	S	L
IVDMD	45.38 0.50±	44.87 0.40±	43.54[c] 0.19±	45.25[b] 0.41±	46.59[a] 0.36±	NS	**
DMI	1.85 0.02±	1.84 0.01±	1.80[b] 0.01±	1.86[ab] 0.02±	1.88[a] 0.02±	NS	**
RFV	65.07 1.36±	64.00 1.08±	60.75[b] 0.62±	65.24[a] 1.38±	67.89[a] 1.19±	NS	*

IVDMD: in vitro dry matter digestibility; DMI: expected dry matter intake; RFV: Relative feed value; Means with horizontally different letters are significantly differed at * (P<0.05); ** (P<0.01); NS = non-significant

With regard to the RFV, which is considered an indicator of the quality of dietary ingredients based on the combination of DM digestibility and the possible consuming rate. In the current study, RFV values of the WS ensiled with the addition of FJLB prepared with the soluble carbohydrates at level of 3 and 5% were increased (P<0.05) to 65.34 and 67.95, respectively compared with 60.98% estimated for samples of WS silage made without the addition of FJLB.

Table 5 shows the effect of interaction between sources and levels of soluble carbohydrates used in preparation of FJLB added at ensiling of wheat straw on some characteristics of nutritive value. The results indicated a significant increase (P<0.05) in IVDMD as a result of ensiling WS with addition of FJLB prepared with soluble carbohydrate regardless to its sources.

Abu-Eloll (2018) observed that ensiling WS with LAB inoculant and increased level of soluble carbohydrates improved IVDMD. The author attributed that improvement to decomposition of plant cell wall components during ensiling as affected by addition of LAB and soluble sugar. Similar result was obtained by Xing (2004) in rice straw silage. Jatkauskas and Vrotniakiene (2006) confirmed the positive role of inoculation with LAB on the digestibility of crude fiber in silage due to the activity of the fiber-degrading enzymes.

Table 5: Shows Effect of interaction between sources and levels of soluble carbohydrates used in preparation of FJLB added ensiling of wheat straw on some characteristics of nutritive value (mean ± SE)

WSC	**Cane- Molasses**			**Date-Molasses**			***P***
Levels	**0**	**3**	**5**	**0**	**3**	**5**	
IVDMD	43.54^{c} 0.29±	45.47^{b} 0.54±	47.14^{a} 0.47±	43.54^{c} 0.29±	45.02^{b} 0.67±	46.05^{ab} 0.44±	*
DMI	1.80 0.02±	1.87 0.03±	1.88 0.04±	1.80 0.02±	1.85 0.03±	1.88 0.01±	NS
RFV	60.75^{b} 0.95±	65.91^{ab} 2.08±	68.70^{a} 2.16±	60.75^{b} 0.95±	64.56^{ab} 2.07±	67.11^{a} 1.19±	*

IVDMD: in vitro dry matter digestibility; DMI: expected dry matter intake; RFV: Relative feed value; Means with horizontally different letters are significantly differed at * (P<0.05); NS = non-significant.

Results showed that RFV were improved when FJLB added at ensiling of WS was prepared with addition of soluble carbohydrates regardless to the source. However, significant (P<0.05) improvement in this estimated criteria was observed with high level of soluble carbohydrates. The RFV values were 68.70 and 67.11% in samples of WS ensiled with addition of FJLB prepared with cane and date molasses at level of 5%, respectively. Since level of cell wall components may affect digestibility and intake (Olomonchi, *et. al.*, 2022). The improvement in RFV in a current study can be explained by lower NDF and ADF contents of WS as a result of ensiling with FJLB prepared with or without addition of soluble carbohydrates.

CONCLUSION

The current study aimed to investigate the effect of ensiling wheat straw with adding FJLB prepared form wild reed plant with different levels of cane or date molasses at ensiling of wheat straw on cell wall components and nutritive value. Results revealed that NDF, ADF and cellulose contents as well as IVDMD, the expected DMI and the RFV of straw were improved with increasing level of those soluble carbohydrates sources. In the same direction, the results showed that the content of ADF and cellulose, in addition to IVDMD and RFV, improved as a result of the interaction effect between the source and level of soluble carbohydrates. The results demonstrate the possibility of using FJLB as an effective alternative to the commercial LAB inoculant as silage additives.

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