



Effect of addition of different levels of commercial inoculant of lactic acid bacteria and soluble sugars on sensory characteristics and chemical composition of wheat straw silages

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Abstract

This study was conducted in Nutrition laboratory to investigate the effect of addition of different levels of Ecosyl, a commercial inoculant of lactic acid bacteria (LAB), 0, 1×10^5 or 1×10^6 cfu/g fresh matter (FM) and four levels of debris as a source of soluble sugars (WSC), 4, 6, 8 or 10% on dry matter (DM) basis on sensory characteristics and chemical composition of wheat straw silages (WSS). Silage samples of 400-500 g were made with 4 replicates after chopping wheat into 1-1.5 cm. Urea was added at rate of 1% to all samples to increase nitrogen (N) content of straw in all silages. Treated samples were packed in nylon bags and ensiled for 60 days. Results revealed that color of WSS samples were ranged between light and dark yellow to greenish yellow. Most inoculated samples were characterized with accepted odor. Results also showed significant ($P < 0.01$) reduction in fibrous components, particularly, neutral detergent fiber from 43.73 to 39.40 and 37.79%, and cellulose from 29.32 to 24.60 and 22.05% were observed in samples of WSS prepared without and with addition of LAB inoculant at level of 1×10^5 and 1×10^6 cfu/g FM respectively. This reduction was correlated with significant ($P < 0.01$) increase in hemicellulose and crude protein (CP) contents. Silages prepared with WSC at level of 10% had lower ($P < 0.01$) contents of acid detergent fiber and cellulose, 37.48 and 23.33% respectively. However, increasing level of debris decreased contents of CP ($P < 0.01$) and ether extract ($P < 0.05$).

Key word: Wheat straw, Ensiling, LAB Inoculant, Chemical composition

تأثير اضافة مستويات مختلفة من اللقاح التجاري لبكتيريا حامض اللاكتيك والسكريات الذائبة في

الخصائص الحسية والتركيب الكيميائي لسايلاج تبين الحنطة

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الخلاصة

اللاكتيك، 0 و 1×10^5 و 1×10^6 وحدة مكونة للمستعمرات/غم مادة رطبة واربعة مستويات من الدبس كمصدر للسكريات الذائبة، 4 و 6 و 8 و 10% على اساس المادة الجافة على الخصائص الحسية والتركيب الكيميائي لسايلاج تبين الحنطة. وقد تم

تحضير نماذج السايلاج 400-500 غم وبأربع مكررات بعد تقطيع تبين الحنطة الى 1-1.5 سم. اضيفت اليوريا الى جميع النماذج بمعدل 1% لزيادة المحتوى النتروجيني في التبن. عبئت النماذج بأكياس مزدوجة من النايلون وحفظت لاهوائيا لمدة 60 يوم. اظهرت النتائج ان لون نماذج سايلاج تبين الحنطة قد تراوحت بين الصفر الفاتح والداكن والاصفر المخضر. وتميزت معظم النماذج المعاملة باللقاح بروائح مقبولة. واطهرت النتائج ايضا حصول انخفاض معنوي ($P<0.01$) في المكونات اللبيفية وبخاصة مستخلص الالياف المتعادل من 43.73 الى 39.40 و 37.79% والسليولوز من 29.32 الى 24.60 و 22.05% في نماذج السايلاج التي حضرت بدون وبإضافة لقاح بكتيريا حامض اللاكتيك بمعدل 1×10^5 و 1×10^6 وحدة مكونة للمستعمرات/غم مادة رطبة على التوالي. وارتبط ذلك الانخفاض بزيادة معنوية ($P<0.01$) في الهيميسليولوز والبروتين الخام. وقد تميزت النماذج التي حضرت بإضافة الدبس بمعدل 10% باقل ($P<0.01$) محتوى من مستخلص الالياف الحامضي والسليولوز، 37.48 و 23.33% على التوالي. الا ان زيادة مستوى اضافة الدبس ادت الى حصول انخفاض في محتوى نماذج السايلاج من البروتين الخام ($P<0.01$) ومستخلص الايثر ($P<0.05$)

Introduction

For economic consideration roughages should consist large portion of ruminant diets. Moreover, these animals may increase their dependence on roughages due to intensification of human demand for arable lands and competition on cereals for food and industry. Wheat straw is the most separated roughages because it is by-produced from the strategic planting of wheat, the main human food all over the world. However, it is characterized with low nutritive value due is to low content of protein and vitamin together with high crude fiber and lignin contents (1). High content of these cell wall components are the main constraint to use these materials in ruminants diets as a results of low digestibility (2).

Ruminants have a unique digestive system in which anaerobic microbes lived in a rumen participate in utilization of low quality roughages, but breaking down complex structure of carbohydrates and increase nitrogen content may help. Many attempts were experimented and actually experienced to ensure that goal. Yet, low palatability of treated straws created great concern to nutritionists, especially, when signs of health to ensure these goals (8). Sources of non-protein nitrogen (NPN) such as urea were

problems on animals fed chemically treated roughages appear (3).

Ensiling is a common practice in many countries to preserve excess forages, to assure the availability of feed throughout the year. In many countries ensiled forages are highly valued as animal feed. In European countries such as Netherland, Germany and Denmark more than 90% of the forages locally produced are stored as silage, even in countries with generally good weather conditions for hay making such as France and Italy about 50% of the forages are ensiled (4). Saeed (5) reported that more palatable crops residuals can be produced by ensiling. Consequently, silage if well done can be a successful alternative (6). Moreover, it may reduce cost of feeding by lowering concentrate levels.

The application of silage additives may improve the fermentation process required to produce well preserved silages. Various additives were tested for enhancing production of lactic acid by stimulation the fermentation process and fast decline in pH (7). Microbial and enzymatic additives in addition to sources of WSC can be applied used to improve N content of ensiled materials and providing silage microbes with

N as well (9). However, in chemical treatment with urea, 70% of the ammonia produced escapes to the environment making this process more expensive and cause environmental pollution (10). Accordingly, this study was conducted to investigate the effect of different levels of inoculant of LAB and debis as a source of WSC together with urea on sensory characteristics and chemical composition of wheat straw silages.

Materials and methods

Preparing of wheat straw silages

Wheat straw (WS) was chopped into 1-1.5 cm and ensiled with different solutions containing 0, 1×10^5 or 1×10^5 cfu/g FM of Ecosyl, a commercial LAB inoculant with 4, 6, 8 or 10% of debis as a source of WSC.

Therefore 12 wheat straw silages (WSS) were prepared. Treatment solutions were diluted with tap water to soak WS, reduce DM content to about 30% and to ensure distribution of additives. Urea was included in all treatment solutions at 1%. Samples of chopped WS were well thoroughly mixed with these solutions and packed in double plastic bags, compacted by hands to exclude air and tightly closed. Plastic bags were then stored in pit silos, covered with soils and kept for 60 days. After that period was passed, silos were opened and plastic bags were moved to laboratory and opened to determine the sensory characteristics and chemical composition. Plan of study was shown in table 1.

Table 1- Plan of the study

LAB inoculant (CFU/g FM)											
0				1×10^5				1×10^6			
4	6	8	10	4	6	8	10	4	6	8	10
WSC (% of DM)											

Determination of sensory characteristics of WSS silages

Sensory characteristics of WSS silage including color, odor and presence of molds were determined according to Saeed and Muhamad (11). Color of samples were depended on yellow, green and brown colors. Description of odor was based on dilution level emitted from samples. Texture was depended on firmness of samples that gave it shape of plastic bags kept in as a die or matrix. Moldiness was referred to by + or – signs representing presence or clearance of molds.

Chemical analysis

Silage samples were analyzed for proximate analysis according to methods of AOAC

(12). Dry matter was determined by drying in air draft oven at 60 °C for 48 hours. Ashing dried samples at 500 °C for 4 hours was used to determine organic matter (OM). Ether extract (EE) was determined by extraction with hexane using Soxhlylate apparatus. Crude protein (CP) was determined by Kjeldahl method using S₄ Kjeltex System. NFE was calculated by difference. Fiber fractions were analyzed according Goering and Van Soest (13). Chemical composition of straw and manures was shown in table 2. Data obtained were analyzed as a factorial experiment in completely randomized design by analysis of variance (14).

Table 2- Chemical composition of wheat straw silages (%) on DM basis

Nutrients	Wheat straw	Urea (NPN)	Debis (WSC)
DM	91.85	-	68.75
Ash	10.85	-	12.23
CP	1.73	287.5 *	2.20
EE	1.48	-	0.69
NDF	72.35	-	-
ADF	46.71	-	-
ADL	31.52	-	-
Cellulose	15.19	-	-
Hemicellulose	25.64	-	-
IVDMD %	39.32	-	-
IVOMD %	41.20	-	-

*46 × 6.25; DM=dry matter; CP=crude protein; EE= ether extract; NDF=neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin; IVDMD=*in vitro* dry matter digestibility; IVOMD= *in vitro* organic matter digestibility

Results and discussion

Sensory Characteristics

Sensory characteristics were determined by sense as soon as plastic bags were delivered to laboratory and opened there. It was noticed that WSS samples were colored with light, greenish or dark yellow. Changes in color may be due to nature and tense of fermentation that ensiled materials exposed to during ensiling (1). In a current study silage color may be affected by increased level of WSC (debis). Caluya (15) reported that color alteration of rice straw silage from yellow to greenish yellow was appeared after 14 days of ensiling and continued till the end of ensiling period (60 days) .

All WSS samples were characterized with accepted, fermented fruit-like odor. This smell was clearly sensed in inoculated WSS. Wet straw-like odor was sensed in other samples especially in those prepared without addition of LAB inoculant and low WSC levels. This agreed with results of Saeed (16), in which fermented fruit-like odor was sensed in molasses-treated wheat straw silage. Existence of organic acid produced from anaerobic fermentation as a result of providing silage microbes with soluble sugars (17). This explanation was supported in the sensory observation of a current study.

In most cases, fermented fruit-like odor or diluted vinegar were sensed in WSS samples prepared with 8 and 10% of WSC levels.

Samples of WSS showed well mass firmness. Saeed and Al-Sultani (18) attributed mass firmness of silages to the type of fermentation, products and compaction of ensiled materials performed during filling the silo. Addition of molasses help producing good texture due to improving fermentation (19).

In some samples, moldiness was existed near the closing points of plastic bags especially in uninoculated WSC samples. Few moldiness was existed in WSS samples prepared with addition of 1×10^5 cfu/g FM. Whereas, those prepared with addition of 1×10^6 cfu/g FM seemed clear of moldiness. These observations can be explained by higher consumption of WSC due to addition and increasing level of inoculation. This may reduce substrate available to aerobic spoilage microbes. Similar finding was observed by Abu-Ellol (20), where concentration of WSC in 4, 6, 8 and 10%-treated wheat straw silages were, 2.10, 1.80, 1.68 and 1.64% of DM. Kaiser, et. al, (2004) demonstrated that silage microbes can utilize WSC. Moreover, higher probable metabolism of WSC by LAB in inoculated

WSS samples may resulted in higher production of lactic acid and other organic acids of fermentation leading to a rapid decrease in pH. These naturally occurring changes may participated in embedding growth of molds and other microbes responsible on aerobic spoilage. The Inhibitory effect of rapid decrease in pH on growth of these microbes was confirmed by many studies (21).

Inoculation ensiled materials with LAB was a common practice all over the world to stimulate lactic acid fermentation, inhibit growth of undesirable microbes and improve preservation (22). At the onset of fermentation, production of lactic acid by homofermentative lactobacilli to decrease pH is preferable to inhibit growth of undesirable microbes such as molds, enterobacteria and aerobic bacteria (23). McDonald, et. al., (24) reported that low numbers of yeasts and molds was an indicator for rapid decrease in pH and exclude of oxygen in laboratory silos.

Characteristics of chemical composition

Table 3 shows effect of addition of different levels of LAB inoculant and WSC on chemical composition of WSS. Statistical analysis revealed that with exception of DM, EE and lignin all nutrients content were significantly affected by level of LAB inoculant. Ash content was increased ($P<0.01$) by 1.42 and decreased ($P<0.01$) by 0.59% due to addition of inoculant at 1×10^5 and 1×10^6 cfu/g FM respectively. Degradation of organic matter of WS during ensiling may explained the increase in ash content. Holzer, et. al., (25) referred to the possibility of degradation of organic components during ensiling at pH condition of 4-5. Decline ash content may be occurred due to improvement of silage fermentation and subsequent decrease in pH during ensiling which lowered degradation of OM as affected by increased level of inoculation. Rapid significant decline in pH of WSS

resulted from higher level of LAB inoculation has been confirmed by Abu-Elol (20). However, Thompson, et. al., (26) reported that higher ash removals were generally, observed at lower pH values.

Results showed that there was a significant ($P<0.01$) increase in CP content in WSS from 3.03 to 3.35% due to inoculation with LAB at 1×10^5 cfu/g FM. This result agreed with that obtained by Li, et al., (7), where ensiling rice straw with inoculation with 1×10^5 cfu/g FM of *L. plantarum* increased content of CP by 0.14-0.62%. Such that increase in CP content has been attributed to the inhibition of undesirable microbes and plant proteases by rapid decline in pH (27). Muck, (21) reported that reduced silage pH conserves WSC and prevents the deamination by inhibiting prolonged fermentation.

Increasing level of inoculation in a current study to 1×10^6 cfu/g FM increased CP content to 3.64%. Additional increase in CP content was associated with lower pH (20). Furthermore, LAB inoculants improve the fermentation quality of silage, which might decrease the degradation of crude protein in the silage; therefore, LAB-treated silage had a high concentration of ruminal ammonia-N. Positive effect of inoculation with LAB on CP content in silages was reported by (28).

Results of present study also showed that ensiling WS with LAB inoculant at 1×10^5 and 1×10^6 cfu/g FM decreased ($P<0.05$) NDF content by 3.32 and 2.71% respectively. A tendency of NDF content of LAB inoculated silage to decrease was observed in another study (29). A significant ($P<0.01$) decrease in ADF content of WSS by 4.33% was noticed in a current study due addition of LAB inoculant at level of 1×10^5 cfu/g FM, higher level of inoculation (1×10^6 cfu/g FM) resulted in additional decrease in ADF content to 6.94%.

Li, et. al., (7) pointed out that use of LAB inoculant decreased NDF and ADF. Similar results were obtained by Nkosi, et. al., (30)

who attributed positive effect of homofermentative LAB inoculant on levels of cell wall fractions to the role of enzymes included in structure of inoculants. However, CF constituents were not affected by inoculation in another study (31). Inconsistency in the effect of inoculation on fibrous content of silage may be due to effect of other factors that probably interfere with the effect of inoculation such as environmental temperature.

According to the changes in NDF and ADF contents of WSS, results revealed that cellulose and hemicellulose contents were significantly ($P<0.01$) affected by the level of LAB inoculant. Low level was associated with a decrease in cellulose by 4.72% and increase in hemicellulose by 2.02%. High level associated with a decrease in cellulose by 7.27 and increase in hemicellulose by 4.24%.

This results agreed with observation of Hapsari, et. al., (32) in which, NDF content was decreased from 62.03 to 59.84% together with an increase in hemicellulose content from 10.22 to 17.26% due to ensiling Napier grass with *L. plantarum* inoculant. Similar results were obtained by Santoso, et. al., (33) in rice straw silage. Increasing numbers of LAB and subsequent increase in its activity may play essential role in these changes.

Nikkhah, et. al., (34) reported that degradation of plant cell walls during ensiling as affected by inoculation with LAB may resulted in a decrease in NDF and ADF contents. Positive effect of inoculation on CF constituents may be enhanced by addition of molasses together with inoculant (35).

Regarding effect of WSC levels on chemical composition of WSS, table 3 showed that there was a slight increase in ash content, yet, it was significantly ($P<0.05$) differed just in WSS prepared with addition of 4 (11.21%) and 6% (11.59%) levels of WSC. This increase was probably resulted from the

accumulative effect of minerals associated with increased levels of WSC. Ash content in debis, the WSC source used in a current study was 12.23%. Similar conclusion was attained by Saeed and Latif (36). However, this result disagreed with that obtained by Sailh (37) who found a decrease by 6.39% in ash content of WSS ensiled for 60 days with addition of molasses at level of 10%. This discrepancy can be explained on basis of higher rate of fermentation may be occurred due to inoculation with LAB by which greater amounts of WSC was utilized as a substrate. According to Rehman (38) WSC is essentially metabolized by LAB to produce lactic acid and maintain good quality fermentation of silage. Moreover, fermentation of WSS in the study of Salih (37) may be prolonged with subsequent prevention of rapid decrease in pH as affected by addition of urea at level of 3%. Addition of urea at ensiling time prolonged fermentation period due to its buffering effect (39). Hence, pH may increases because of additional lactic acid required to equalize the buffering effect on fermentation.

Crude protein (CP) content was also increased ($P<0.01$) in a current study with increasing level of WSC from 4 to 6 and 8. Similar result was obtained by Saeed and Muhamad (11) in ensiled corn cobs due to increasing level of debis from 4 to 10%. Since WSC is very essential to support growth of LAB and fermentation of good quality (40). Increased CP may be due to role of soluble sugars in debis in improving silage fermentation. Then, reducing losses in nutrients including CP (41). With higher level of WSC source (10%) the increase in CP content in WSS obviously shown was retarded and being close to that of WSS ensiled with WSC at level of 4%. This in turn may be due to increased DM loss. Abu-Ellul (20) reported that increasing WSC levels increased DM loss in WSS. Similarly, Saeed (16) found that increasing level of

Table 3- effect of level of LAB inoculant and source of WSC on chemical composition of WSS (% \pm SE)

Nutrients	LAB inoculant (CFU*/g FM)			WSC (%)				P	
	0	1×10^5	1×10^6	4	6	8	10	LAB	WSC
DM	29.34 \pm 0.10	29.25 \pm 0.10	29.20 \pm 0.11	29.50 \pm 0.09	29.19 \pm 0.11	29.25 \pm 0.15	29.11 \pm 0.11	NS	NS
Ash	11.24 ^b \pm 0.18	12.42 ^a \pm 0.07	10.65 ^c \pm 0.06	11.21 ^b \pm 0.21	11.59 ^a \pm 0.26	11.53 ^{ab} \pm 0.22	11.43 ^{ab} \pm 0.23 \pm	**	*
CP	3.06 ^c \pm 0.03	3.35 ^b \pm 0.03	3.64 ^a \pm 0.05	3.22 ^b \pm 0.05	3.45 ^a \pm 0.08	3.45 ^a \pm 0.08	3.29 ^b \pm 0.08	**	**
EE	2.33 \pm 0.03	2.33 \pm 0.09	2.41 \pm 0.05	2.52 ^a \pm 0.10	2.39 ^{ab} \pm 0.04	2.19 ^b \pm 0.05	2.33 ^b \pm 0.05	NS	*
NDF	65.89 ^a \pm 1.03	63.57 ^{ab} \pm 0.84	63.18 ^b \pm 0.75	65.65 ^a \pm 1.26	64.89 ^a \pm 1.04	64.34 ^{ab} \pm 0.73	61.98 ^b \pm 0.96	*	*
ADF	43.73 ^a \pm 0.80	39.40 ^b \pm 0.78	36.79 ^c \pm 0.76	41.36 ^a \pm 1.31	40.32 ^a \pm 1.35	40.73 ^a \pm 0.54	37.48 ^b \pm 1.08	**	**
ADL	14.40 \pm 0.24	14.75 \pm 0.17	14.73 \pm 0.24	14.87 \pm 0.20	14.70 \pm 0.18	14.86 \pm 0.24	14.08 \pm 0.34	NS	NS
Cellulose	29.32 ^a \pm 0.07	24.60 ^b \pm 0.81	22.05 ^c \pm 0.91	26.48 ^a \pm 1.30	25.62 ^a \pm 1.38	25.87 ^a \pm 0.68	23.33 ^b \pm 1.16	**	*
Hemicellulose	22.15 ^b \pm 1.07	24.17 ^{ab} \pm 0.70	26.39 ^a \pm 0.79	24.28 \pm 1.34	24.56 \pm 1.23	23.60 \pm 0.84	24.50 \pm 1.11	**	NS

*CFU/ Colony forming unit

DM=dry matter; CP=crude protein; EE= ether extract; NDF=neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin

Means having different letters at the same row are significantly different at * (P<0.05) ** (P<0.01)

molasses from 0, 5, 10, 15, 20, 25 and 30% associated with a decrease in CP content of WSS from 3.26 to 3.17, 3.26, 3.02, 2.76 and 2.50% respectively. In spite of similar trend between the last and a current study but a difference was noticed in the level of WSC source in which a decrease in CP content of WSS has been appeared. In a current study a decrease started at 10%, whereas, higher than 10% was the level of WSC in a study of Saeed (16). This difference may be due to source of WSC (debris vs. molasses), and/or role of LAB inoculant added in a current study, where, CP content was increased with higher level of inoculant.

Results revealed that ether extract content of WSS was slightly decreased ($P<0.05$) with increasing WSC levels from 4 and 6% to 8 and 10%, EE contents were 2.52, 2.39, 2.19 and 2.33% for these levels of WSC respectively. Similar result was reported by Alikhani, et. al., (42). Ebrahim Shahraki and Saravani (43) attributed such changes to dilution effect with molasses containing low content of EE.

Although, EE content was decreased with increasing WSC levels, ensiling process per se increased EE content of 1.48% in WS. This observation can be explained by production of volatile fatty acids (VFA) from oxidation of WSC during anaerobic fermentation of silage. Saeed and Muhamad (11) reported that providing silage microbes with increased amounts of WSC such molasses or date honey increased VFA concentration.

Increasing WSC level was shown to decrease NDF and ADF contents in WSS. Lower ($P<0.05$) content of NDF (61.98%) and ADF (37.48%) were observed in WSS ensiled with higher level of WSC. The reason for that may be the partial degradation of complex mass of carbohydrates present in WS by the increased activity of silage microbes as it provided with a source of degradable protein energy.

Baytok, et. al., (44) referred to the degradation of cell wall constituents as affected by stimulation of silage fermentation due to addition of molasses. Inconsistently, Saeed (16) reported that addition of molasses at level of 10% decreased CF content of WSS by 1.94% as compared with addition molasses at level of 5% .

The decrease in WSS contents of NDF and ADF in a current study can also be explained by the dilution effect of WSC source which has undetectable content of these insoluble carbohydrates. This seemed in line with observation of Babaeinasab, et. al., (35) in which NDF and ADF contents of wheat-potato silage were decreased as a result of addition of molasses, it was attributed to dilution effect of molasses containing low content of CF.

Cellulose content in WSS was also affected ($P<0.05$) by addition of different levels of WSC. Lower cellulose content was observed in WSS samples ensiled with WSC at level of 10% as compared with those ensiled with 4, 6 and 8% levels of WSC. Cellulose contents in WSS ensiled with these four levels were, 23.33, 26.48, 25.62 and 25.87% respectively. The fitness of pH values in WSS as affected by increased WSC levels arranged good condition for bacterial activity to attack fibrous mass in WSS resulting in a decrease in cellulose content. Similar idea was proposed by Castro, et. al., (44).

Regarding effect of interaction between level of LAB inoculant and WSC, results showed that DM content was significantly ($P<0.01$) by this interaction. Higher value (29.29%) was recorded in WSS prepared with 1×10^5 cfu/g FM of inoculant and 4% of WSC source, whereas, lower DM content (28.85%) was recorded in WSS prepared with inoculant and WSC at levels of 1×10^6 cfu/g FM and 8% respectively (table 4). Similar observation were attributed by Abu-

Table 4- Effect on interaction between level of LAB inoculant and WSC source on chemical composition of wheat straw silages (% \pm SE)

LAB level	0				1×10^5 cfu/g FM				1×10^6 cfu/g FM				P
WSC level %	4	6	8	10	4	6	8	10	4	6	8	10	
DM	29.63 ^{ab} ± 0.08	29.11 ^{bcd} ± 0.15	29.62 ^{ab} ± 0.22	29.01 ^{bcd} ± 0.22	29.75 ^a ± 0.06	28.92 ^{cd} ± 0.22	29.29 ^{abcd} ± 0.12	29.03 ^{bcd} ± 0.21	29.12 ^{bcd} ± 0.15	29.54 ^{abc} ± 0.13	28.85 ^d ± 0.33	29.31 ^{abcd} ± 0.15	**
Ash	10.58 ^d ± 0.03	11.09 ^{cd} ± 0.45	11.69 ^{bc} ± 0.21	11.63 ^{bc} ± 0.39	12.31 ^a ± 0.10	12.78 ^a ± 0.09	12.44 ^a ± 0.07	12.17 ^{ab} ± 0.12	10.74 ^d ± 0.08	10.90 ^d ± 0.07	10.48 ^d ± 0.10	10.49 ^d ± 0.12	**
CP	3.13 ^c ± 0.06	3.09 ^{cd} ± 0.05	3.10 ^{cd} ± 0.11	2.91 ^d ± 0.04	3.14 ^c ± 0.10	3.42 ^b ± 0.01	3.41 ^b ± 0.01	3.43 ^b ± 0.01	3.39 ^b ± 0.02	3.84 ^a ± 0.03	3.83 ^a ± 0.07	3.51 ^b ± 0.10	**
EE	2.43 ± 0.04	2.36 ± 0.05	2.24 ± 0.08	2.29 ± 0.08	2.60 ± 0.32	2.39 ± 0.06	2.09 ± 0.06	2.23 ± 0.11	2.53 ± 0.11	2.42 ± 0.10	2.24 ± 0.10	2.46 ± 0.05	NS
NDF	68.47 ± 2.64	67.58 ± 2.37	63.50 ± 1.36	64.00 ± 1.18	64.56 ± 2.09	64.44 ± 0.75	65.97 ± 1.38	59.33 ± 0.64	63.91 ± 1.52	62.66 ± 1.46	63.55 ± 0.92	62.61 ± 2.27	NS
ADF	46.85 ^a ± 0.76	46.43 ^a ± 1.14	41.02 ^{bc} ± 0.90	40.63 ^{bc} ± 1.04	40.12 ^{bc} ± 1.65	39.66 ^{bcd} ± 0.03	41.94 ^b ± 0.52	35.90 ^{de} ± 1.94	37.11 ^{cde} ± 1.56	34.89 ^e ± 1.05	39.25 ^{bcd} ± 1.04	35.91 ^{de} ± 1.91	**
ADL	14.85 ± 0.48	14.82 ± 0.46	14.27 ± 0.10	13.67 ± 0.67	15.27 ± 0.22	14.59 ± 0.19	14.84 ± 0.53	14.32 ± 0.31	14.49 ± 0.29	14.70 ± 0.31	15.48 ± 0.35	14.26 ± 0.79	NS
Cellulose	31.99 ^a ± 0.33	31.61 ^a ± 1.42	26.74 ^{bc} ± 1.01	26.96 ^{bc} ± 0.52	24.85 ^{bcd} ± 1.73	25.06 ^{bcd} ± 0.17	27.10 ^b ± 1.06	21.41 ^{de} ± 2.09	22.62 ^{cde} ± 1.68	20.19 ^e ± 1.32	23.76 ^{bcd} ± 1.07	21.64 ^{de} ± 2.15	*
Hemicellulose	21.62 ± 2.84	21.15 ± 2.42	22.48 ± 1.82	23.36 ± 1.96	24.44 ± 1.69	24.78 ± 0.74	24.02 ± 0.89	23.43 ± 2.20	26.79 ± 2.19	27.76 ± 2.01	24.30 ± 1.71	26.70 ± 1.55	NS

*CFU/ Colony forming unit

DM=dry matter; CP=crude protein; EE= ether extract; NDF=neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin

Means having different letters at the same row are significantly different at * (P<0.05) ** (P<0.01)

Ello1 (20) to the nature of silage fermentation which may be enhanced by high level of inoculant as evidenced by lower pH associated with high introduction of lactic acid by LAB, the beneficial microbes. Higher production of lactic acid in a current study can be proposed according to increased numbers of LAB associated with addition of inoculant and increasing its level, which in turn utilized the available soluble carbohydrates with lower DM loss. Babaeinasab, et. al., (35) reported that utilizing WSC by LAB may reduce DM loss. Results revealed that ash content of WSS was significantly ($P < 0.01$) affected by interaction effect. Higher content (12.78%) was observed in WSS prepared with 1×10^5 cfu/g FM of inoculant and 6% of WSC source, whereas, lower DM content (10.48%) was recorded in WSS prepared with inoculant and WSC at levels of 1×10^6 cfu/g FM and 8% respectively. The increase in ash content may be due to degradation of organic matter during ensiling. Holzer, et. al., (25) confirmed degradation of some organic components during fermentation process at pH condition of 4-5. Yet, the decrease in ash content may be due to an improvement in silage fermentation and subsequent decrease in degradation rate of OM associated with rapid decline in pH. This explanation is in line with observations of Thompson, et. al., (26) in which removals of ash were increased at lower pH values.

Content of CP in WSS was significantly ($P < 0.01$) affected by this interaction too. Higher content (3.84%) of CP was recorded in WSS prepared with 1×10^6 cfu/g FM of inoculant and 6% of WSC source, but, lower content (2.91%) was recorded in WSS prepared without inoculant and WSC at level of 10%. This may be due to inhibition of undesirable microbes and plant proteases by lower pH associated with inoculation with LAB (7). Similar findings and conclusion were derived by Abu-Ellol

(20). Cao, et. al., (41) reported that addition of LAB inoculant improved quality of silage fermentation and decreased protein degradation in ensiled materials.

Results of a current study also showed that ADF content was significantly ($P < 0.01$) affected by interaction between level of inoculant and WSC source. Lower content (34.89%) was recorded in WSS prepared with 1×10^6 cfu/g FM of inoculant and 6% of WSC as compared with ADF content of 46.85% in WSS prepared without inoculant and 4% level of WSC. This decrease in ADF content may be resulted from stimulation of cell wall degradation by enhanced fermentation due to increased number of LAB. Li, et. al., (2010) observed that the ensiling with LAB inoculant decreased ADF content of wheat straw.

Cellulose content of WSS was also significantly ($P < 0.05$) affected by the mentioned interaction effect. Lower content of cellulose (20.19%) was recorded in WSS prepared with 1×10^6 cfu/g FM of inoculant and 6%, whereas, higher compared content (31.99%) was observed in WSS prepared without inoculant and lower level of WSC. Increased numbers and activity of LAB seemed to play essential role in these changes. Degradation of plant cell walls during ensiling can be lead to a decrease in ADF content of ensiled whole corn plant inoculated with LAB (34).

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