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EFFECTS OF SILAGE EFFLUENT, UREA-MOLASSES AND WATER TREATMENT ON *IN SITU* RUMEN DEGRADATION AND MICROBIOLOGICAL TRAITS OF WHEAT STRAW

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ABSTRACT

This experiment was carried out to determine the effects of silage effluent, urea-molasses and water treatment on *in situ* disappearance of dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) of wheat straw, and its degradation parameters (a + b + c and a+b) and effective degradability (ED). There were six treatments: including control (W), W+ silage effluent (WS), W+ silage effluent + water + urea + molasses (WSHUM), W+ urea + molasses + water (WUMH), W+ urea + molasses (WUM) and W+ water (WH). Except control and WH groups, all other groups were set iso-caloric and iso-nitrogenous and calculated amount of urea and molasses equal to nitrogen and energy value from silage effluent were added to treatment groups. All treatment groups were packed in sealed plastic bucket made by polypropylene, and they were subjected to 45 days of anaerobic fermentation. Four rumen cannulated Holstein bulls of 400 kg and 20 months of age were utilized. The extent and rate of DM, OM, CP, ADF and NDF disappearance was determined by using randomized block design where animals were blocking factor. After fermentation, all treatments were incubated to rumen for 4, 8, 16, 24, 48, 72 and 96 h using nylon bag technique. Data of *in situ* DM, OM, CP, ADF and NDF degradability were subjected to one-way ANOVA using the GLM procedure. A completely randomized design was used in which treatment groups were the fixed factor. Differences in terms of DM, OM, CP, ADF and NDF degradabilities among the treatments were significant ($p \leq 0.05$). Treatment with silage effluent increased rumen degradability of wheat straw ($p \leq 0.05$). Also, treating wheat straw with silage effluent hindered the growth of pathogenic microorganisms. In conclusion, treating low quality roughages i.e. wheat straw with silage effluent which is rich in nutrient could be recommended not only to improve fermentation quality of wheat straw but also to increase its degradation in rumen. Further, addition of silage effluent can preserve the ensilage from undesired pathogen i.e. yeast, coliform, clostridia and fungal growth.

Keywords: *in situ* degradability; ADF; NDF; Silage effluent; Wheat straw.
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INTRODUCTION

Wheat straw, a major agricultural residue, is abundantly produced throughout the world. On an average one kg wheat grains produce 1.2–1.3 kg of straw (Dai *et al*., 2016). In Turkey, approximately 29.8 million tons wheat straw is being produced annually (Ak and Akbay, 2018). Most of this by-product is fed to animals. It contains lignocellulosic material including 35-37% cellulose, 25% hemicellulose, 10–15% lignin, and 5–10% mineral and other components (Merali *et al*., 2013; Liu *et al*., 2014). These cell wall structures are less available for microbial degradation in rumen. Hence, less energy and low protein contents make it less favorable to use as feed stuff (Yang *et al*., 2006; Chaturvedi and Verma, 2013).

Previously, treatment of wheat straw with chemicals (Chandra *et al*., 2012; Chaturvedi and Verma, 2013; Li *et al*., 2013), fungal enzymes (Van Kuijk *et al*., 2015), and lactobacillus bacteria (Ni *et al*., 2014) has been employed to improve digestibility. Silage effluent, an agricultural pollutant, is abundantly generated worldwide. This environmental pollutant liquid is rich in lactobacillus bacteria with acidic pH (3.24-4.5) and 6-10% dry matter. Effluent's contains 19-33% CP, 40-42% water soluble carbohydrates (WSC) and 15-25% ash on a dry matter (DM) basis (Patterson *et al*., 1979; Gebrehanna *et al*., 2014; Yildiz *et al*., 2016). The amount of effluent produced varies between 5 and 20% depending on DM of ensiled crop (Gebrehanna *et al*., 2014), extent of chopping and pressing (Johnson *et al*., 2002; Holmes and Muck, 2007), use and type of additives (Yitbarek and Tamir, 2014), and pressure applied on surface (Alli *et al*., 1985). Disposal of effluent is major constraint in livestock production. In previously studies, silage effluent has been fed to pigs and dairy cattle (Patterson *et al*., 1979; Randby, 1997), and used for soil treatment (Yildiz *et al*., 2016). However, no standard method has been developed for its disposal or effective use. Lactobacillus (LAB) inoculant along with WSC source has been successfully used to improve fermentation quality of wheat straw (Ni *et al*., 2014), but its effect on digestion has not been studied. The aim of

this experiment was to determine the nutrient composition of silage effluent and its effect on *in situ* degradation of wheat straw by using *in situ* nylon bag technique. Furthermore, the comparative degradation parameters of other treatments were also studied.

MATERIALS AND METHODS

Treatments: Wheat straw (Triticum aestivum cv. Barbela) was obtained from Research and Application Farm of Agriculture Faculty, Ataturk University, Erzurum. Silage effluent was provided from corn crop after fermentation ensiled at this Research Farm in October 2016 (Yildiz *et al*., 2016). Before mixing, chemical and microbial analysis of wheat straw and effluent were performed (Table 1). Six treatment groups were established: 1. control (W), 2. straw + urea-molasses (WUM), 3. straw + water (WH), 4. straw + water + urea-molasses (WHUM), 5. straw + silage effluent (WS), and 6. straw + 50% silage effluent + 50% water + urea-molasses (WSHUM) (Table 2). Required amount of effluent and water was added, and dry matter content in WH, WHUM, WS and WSHUM was kept 40%. Except for control and WH groups, all other groups were set iso-caloric and iso-nitrogenous and calculated amount of urea and molasses equal to nitrogen and energy value from silage effluent (Table 1) was added to treatment groups. All treatment groups were packed in sealed plastic bucket made by polypropylene and allowed fermentation for 45 days. After fermentation, the fermented straws were dried and ground to 2-2.5 mm before rumen incubation.

Physical and Microbial Analysis: After 45-day fermentation, the quality of ensiled treatment groups with respect to color, odor and purification (moldiness) was analyzed (Kılıç, 1986; Filya, 2000). Microbial analysis including total bacterial count, lactobacillus (LAB) and coliform bacteria, yeast and mold of treatment groups and silage effluent was performed (Seale *et al*., 1990).

Chemical Analysis: Dry matter, crude protein (CP), ether extract, WSC of silage effluent, 45-day fermented treatment groups and rumen incubated residues were measured (AOAC, 1990). Fiber analysis (Van Soest *et al*., 1991) was done using Ankom Fiber Analyser 2000 (ANKOM Technology, Macedon, NY). Heat stable amylase was used to determine neutral detergent fiber (aNDFom). Acid detergent fiber (ADFom) and other fiber fractions are expressed excluding residual ash.

Animals and Diets: To determine *in situ* degradation of treated wheat straw, four rumen cannulated Holstein bulls of 400 kg and 20 months of age were housed individually on concrete floor and fed according to 1.25x maintenance (NRC 2001) ration containing 55% concentrate, 20.5% dried grass, 20.5% *alfalfa* hay and 4% wheat straw.

The ethical approval for this experiment was taken from Local Ethics Committee of Animal Experiment, Ataturk University, Erzurum, Turkey.

***In situ* Rumen Incubation:** The extent and rate of DM, OM, CP, ADF and NDF disappearance was determined by using randomized block design where animals were blocking factor. Five g sample from each treatment, in duplicate, was taken in nylon bags measuring 10 x 15 cm (length and width) with the pore size of 50-60 µm. The bags closed and tied with nylon finishing line were incubated in the rumen for 4, 8, 16, 24, 48, 72 and 96 hours and removed at same time. After removal the bags were soaked in cold water and then washed with running tab water till rinse was clear. To determine 0 h disappearance (washing loss) duplicate bags from each sample were taken into bag but without hanging in rumen washed with running tab water as described above (Jalilvand *et al*., 2008). After room aeration bags were dried at 55°C for 48 hours and weighed, and residues were ground to 1 mm and saved for later analysis.

Calculations: The disappearance of DM, OM, CP, ADF and NDF for each incubation time were calculated from the proportion remaining post rumen incubation. This rumen disappearance rate was fitted into following exponential model of Ørskov and McDonald (1979), and a, b and c values of each treatment group were estimated using NEWAY program (McDonald, 1981). Apparent degradability was denoted by a + b (upper asymptote).

$$P = a + b\,(1-e^{-ct}) \quad (1)$$

where 'P' is the disappearance at time 't', 'a' represents washing loss (or quickly degradable fraction), 'b' denotes slowly degradable fraction and 'c' is constant rate of degradation of 'b' (Palangi and Macit, 2019). Effective degradability (ED) was calculated using following equation (Ørskov and McDonald, 1979).

$$ED = a+[bc/(c+k)] \quad (2)$$

where 'a' 'b' and 'c' are the constants as described earlier in the non-linear equation above and 'k' the rumen fractional outflow rate (0.02/h, 0.05/h, 0.08/h).

Statistical Analysis: Data were analyzed under completely randomized design using a General Linear Model (GLM) procedure of SAS (SAS Institute Inc. 2004), Duncan (1955)'s multiple range test was used for the comparison of means. Feeds were the only sources of variation considered. Following mathematical model was assumed for statistical analysis.

$$Y_{ij}=\mu+T_{i}+e_{ij}$$

where; Y_{ij} represents dependent variable; µ is general mean; T_i is effect of treatment (i= 6); e_{ij} is random error associated with j^{th}. observation on i^{th} treatment.

RESULTS AND DISCUSSION

Ensiling wheat straw after 5 different treatments showed difference in color and smell (Table 3). Based on color, smell and moldiness; straw containing silage effluent was of good quality with typical fruity smell and brownish yellow (olive drab) color. WH and WHUM were moldy and WH group was worst with putrefying smell and blackish brown color.

After 45-day fermentation, both WS and WSHUM silages were of good quality containging10^8 (cfu g-1 LAB) (Table 3). This result fulfils the criteria reported by (Zielinska *et al*., 2015) that during fermentation silages are preserved when LAB reaches at least 10^5 (cfu g-1). Improvement in fermentation quality of WS and WSHUM groups can be explained according to the Yitbarek and Tamir (2014) where explained that addition of sugar and LAB inoculant increased fermentation. Inoculation of lactobacillus bacteria of effluent lowered pH by converting the WSC into organic acids and restricted the growth of mold, coliform and other pathogenic bacteria (Ni *et al*., 2014; Zielinska *et al*., 2015). Straw fermented with urea-molasses and 60% moisture content (WHUM) had better quality and more LAB than that which had no moisture but urea-molasses (WUM). These results agreed to the findings (Ni *et al*., 2014) which proved the importance of moisture during fermentation. Straw ensiled with water (WH) was putrefied, and had layer of mold and high pathogenic bacteria (Table 3). The poor fermentation of straw without sugar additive (WH) has also been reported by Yitbarek and Tamir (2014) where sugar proved as a limiting factor in good quality fermentation.

Chemical composition of all treatment groups after 45-day fermentation had variable CP, ash, CF, ADF and NDF contents, but there was no significant variation in DM content of all treatments (Table 4). An increase in CP contents was noted in all treatments compered to control group. WSHUM and WUM had the highest CP while it was similar for WHUM and WS groups.

No research work on chemical composition of effluent treated wheat straw has so for been conducted elsewhere, so the published data on this aspect is not available for comparison. However, it can be explained in a way that, presence of WSC and lactobacillus may have increased fermentation by decreasing pH in effluent treated silages. Most probably this decrease in pH helped NH3 to make bond with fibrous material by changing it into free ionic form as previously described (Oladosu *et al*., 2016). Similarly, trapping of free ammonia by organic acids has also been reported earlier (Borhami *et al*., 1982; Elobied *et al*., 2013). The higher CP content of silage treated with water than control reinforce the views of (Seale *et al*., 1986) that presence of E.coli and clostridia bacteria in putrefied silages may cause higher N concentration. Ash contents of both silage effluent containing (WS, WSHUM) groups increased compared to control group but for other groups no big variation was noted. There was a decrease in crude fibers, ADF and NDF contents of silage effluent treated groups while it was higher for WH, WUM, WHUM groups than control. The reduction in NDF and ADF contents of WS and WSHUM during fermentation was due to hydrolysis of fiberous material caused by microbes as previously documented (Ni *et al*., 2014; Oladosu *et al*., 2016). Mahr-un-Nisa *et al*. (2004) confirmed that straw treatment with urea and corn steep liquor increased NDF and ADF contents by increasing neutral detergent insoluble nitrogen (NDIN) concentration. They explained that most of N produced during fermentation of urea-carbohydrate (WSC) treated straw, held in form of NDIN which results in NDF and ADF increased concentration. In present study, urea-molasses treatment (WUM, WHUM) increased NDF and ADF contents of ensiled wheat straw. The entire increase in NDF and ADF in WUM and WHUM was due to fiber-N bond. These results are in convenience with the findings of other researchers (Brown *et al*., 1987).

The pattern of DM, OM, CP, ADF and NDF disappearance during 0, 4, 8, 16, 24, 48, 72 and 96 hours rumen incubation has been illustrated in Fig 1-5. The disappearance of DM, OM, CP, ADF and NDF for almost all incubation periods was higher in the WUM, WHUM, WS and WSHUM groups than the control group ($p \leq 0.05$). Despite same nutrient loss from nylon bag at 0 h, the DM, OM, CP, NDF and ADF disappearance of WHUM was lower than WS. The disappearance of DM, OM, CP, ADF, and NDF for almost all incubation periods was higher for silage effluent treatments ($p \leq 0.05$). The loss of OM, ADF and NDF at 96 h for WH was lower than control ($p \leq 0.05$), while DM and CP disappearance for same groups was not different from control group ($p > 0.05$). The disappearance of DM of urea-molasses treated wheat straw increased significantly ($p \leq 0.05$). These results were in agreement to the findings of some other workers (Gupta *et al*., 1977; Dass *et al*., 2000). The disappearance of CP and ADF was high for WS group while NDF disappearance was high for WSHUM group. Similarly, increase in rumen disappearance of CP, NDF, ADF due to urea treatment has also been reported by (Dass *et al*., 2000). No significant difference was found in DM and OM disappearance of WS and WSHUM ($p > 0.05$). WHUM had more nutrient loss than WUM group ($p \leq 0.05$). The difference between WUM and WHUM degradation could be attributed to higher fermentation in later group due to presence of moisture and energy. These results from present study were within the observations of most of the earlier studies (Gupta *et al*., 1977; Ni *et al*., 2014). The sugar is a limiting factor during fermentation and lactobacillus use sugar for fermenting ensilage material (Gupta *et al*., 1977; Seale *et al*., 1990). Therefore, wheat

straw ensiled with water without adding sugar (WH) fermented poorly. Presence of coliform and other pathogen bacteria (clostridia) in silages are generally considered detrimental (Zhang *et al*., 2017). In present study, negative effect of water treatment (WH) on rumen degradation could be due to presence of anaerobic pathogens which had restrict rumen microflora from digestion.

The ensiling of wheat straw with silage effluent increased the ruminal disappearance of DM, CP, NDF and ADF almost for every incubation time. Both WS and WSHUM had higher nutrient loss in rumen at 96 h than other treatment (Fig. 1-5). This increase in rumen degradation may be due to physio-chemical changes occurred in the cell wall during microbial fermentation as described by Zorrilla-Rios *et al*., (1985) and Mahr-un-Nisa *et al*.,–(2004). They reported that NH3 produced during fermentation increases the fragility of wheat straw. Increase in fragility of cell wall due to microbes has also been documented (Ni *et al*., 2014). In present study, linkage between lignin-cellulose or lignin-hemicellulose may have been hydrolyzed by enzymes, released during microbial fermentation. However, degradation pattern of silage effluent treated straw can not be compared with previous literature as almost no published data related to this aspect is available.

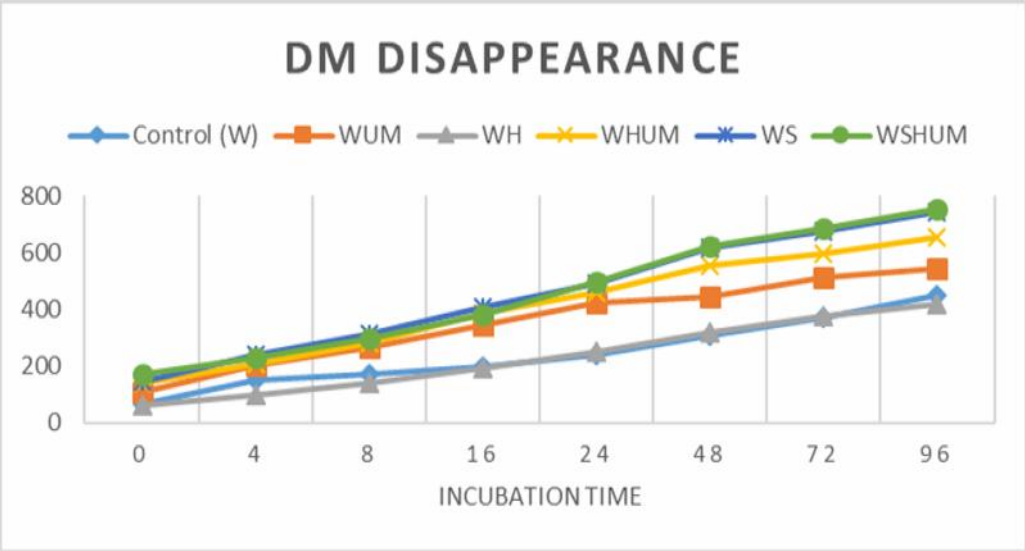
The estimates of a, b, c and a + b for control and treated groups of wheat straw are given in table 5. There was large variation in "a" fractions among all groups ($p \leq 0.05$). Rapidly degradable "a" fraction of all nutrients was highest for both silage effluent treatments ($p \leq 0.05$). Water treatment had lowest "a" fraction for DM, OM and NDF ($p \leq 0.05$). However, "a" fraction in control group for CP and ADF was lowest ($p \leq 0.05$). Straw ensiled with urea-molasses had less "a" fraction of all nutrients than that treated with urea-molasses and water ($p \leq 0.05$).

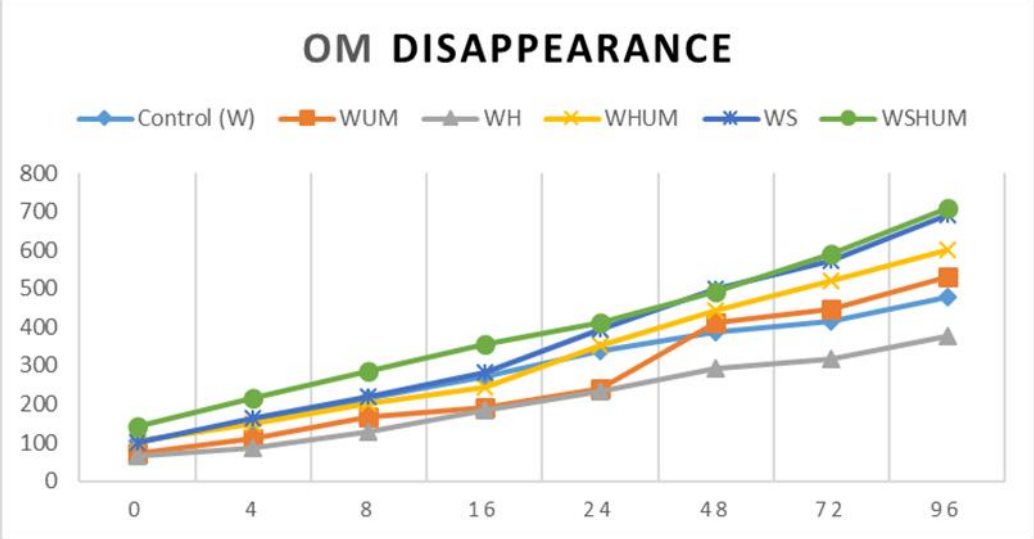
The slowly degradable fractions "b" of DM, OM, CP, ADF and NDF were greatest for both silage effluent (WS, WSHUM) treatments ($p \leq 0.05$). The difference of "b" fraction between control and water treated straw (WH) was significant for DM, CP and NDF ($p \leq 0.05$) and non-significant for OM and ADF ($p > 0.05$). There was large variation between WUM and WHUM treatment groups in terms of "b" fractions. This fraction of WHUM was high for DM and NDF and low for CP than WUM ($p \leq 0.05$) while this "b" fraction of OM and ADF was similar between these two groups ($p > 0.05$).

There was significantly variation in degradation rate of all treatment groups. Control group had faster degradation rate of OM and ADF while WUM group had highest "c" fraction of DM and CP ($p \leq 0.05$) (Table 5). Differences in degradation rate of NDF among the WUM, WH and WHUM were insignificant ($p > 0.05$), and but these feed groups had higher NDF values than those of other treatment groups ($p \leq 0.05$).

Effective degradability (ED) values decreased consistently with the increase in rumen outflow rates (Table 5). At all three rumen outflow rates (0.02/h, 0.05/h, 0.08/h), ED values of OM and CP were highest in WSHUM, for ADF it was high in WS group ($p \leq 0.05$) while for DM and NDF, this ED value was significantly same ($p > 0.05$) in both WS and WSHUM (Table 5). ED of OM and ADF was lowest ($p \leq 0.05$) for water treated straw while difference among ED values of DM, CP and NDF for control and WH groups was not significant ($p > 0.05$).

Though some studies related to the effects of silage effluent, urea-molasses and water treatment on *in situ* disappearance of dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) of wheat straw, there have been no reports about the effects of mentioned treatments on the degradation parameters (a+b+c and a+b) and effective degradability (ED) of wheat straw. Hence, current study was carried out to determine the effects of silage effluent, urea-molasses and water treatment on the degradation parameters (a + b + c and a+b) and effective degradability (ED) of wheat straw. However, degradation parameters and effective degradability of silage effluent treated straw may not be compared with previous literature because there have been no published article related to this aspect is available.


DM DISAPPEARANCE
Control (W)
WUM
WH
WHUM
WS
WSHUM
800
600
400
200
0
0
4
8
16
24
48
72
96
INCUBATION TIME


OM DISAPPEARANCE
Control (W)
WUM
WH
WHUM
WS
WSHUM
800
700
600
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300
200
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0
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4
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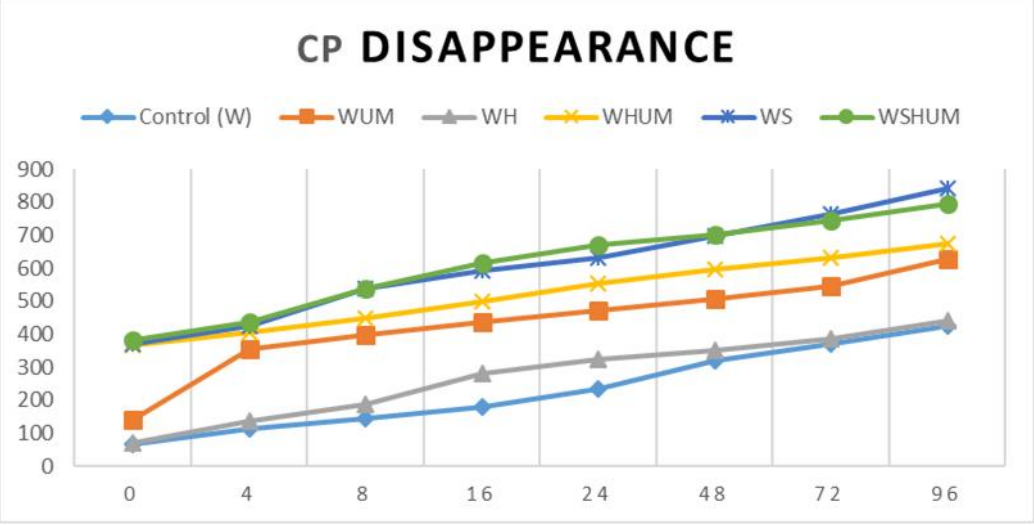
CP DISAPPEARANCE
Control (W)
WUM
WH
WHUM
WS
WSHUM
900
800
700
600
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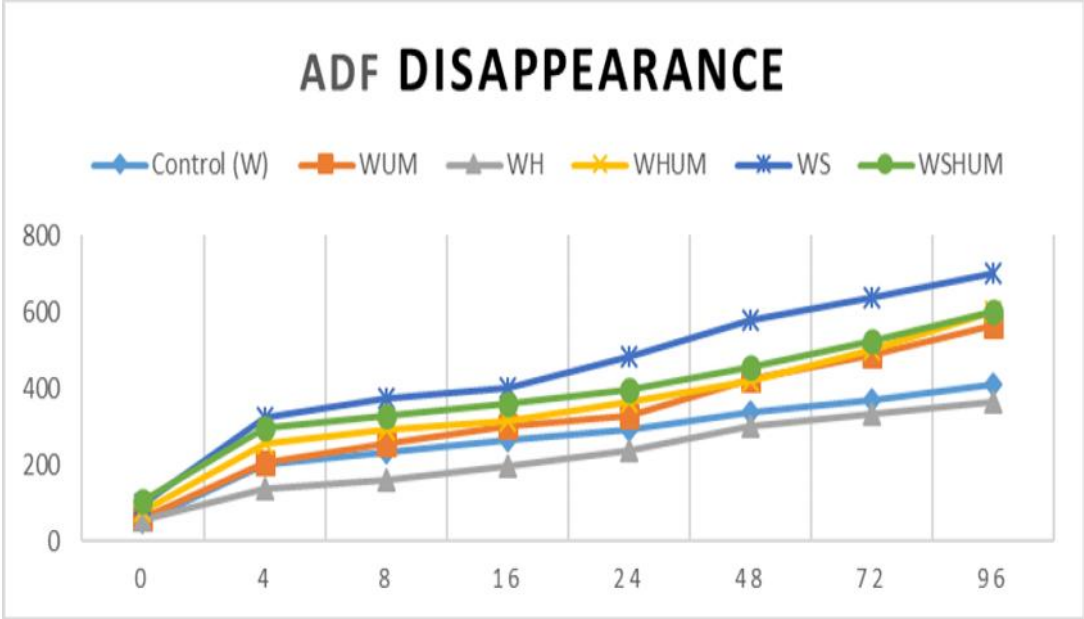

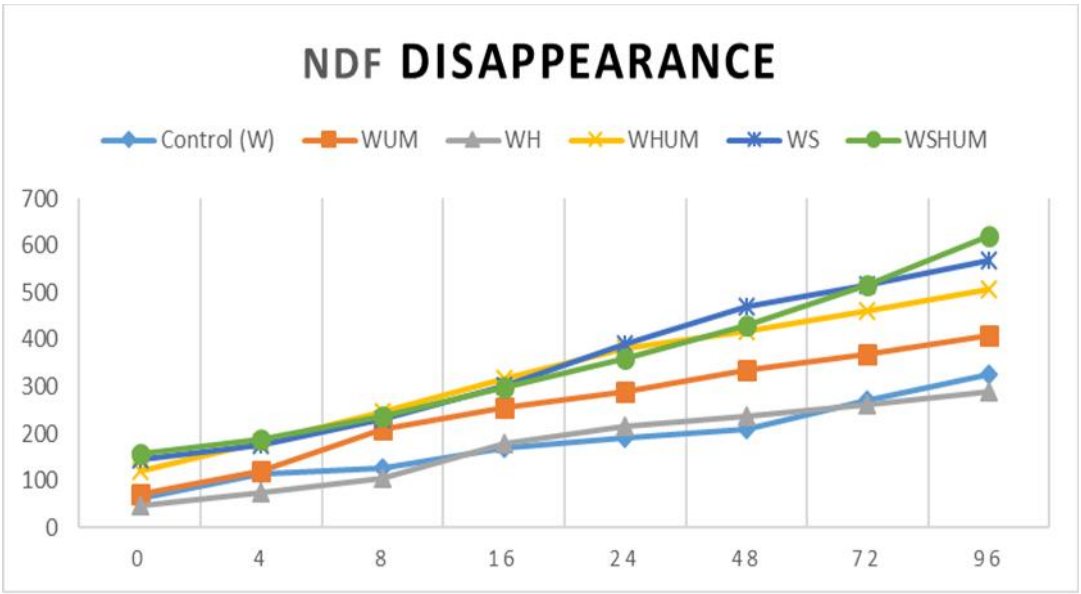

Table 1. Chemical compositions of wheat straw and silage effluent.

Wheat Straw (W)		**Silage Effluent (S)**	
Dry Matter (DM) (g/kg)	924	Dry Matter (DM) (g/kg)	120
Nutrient composition (g/kg DM)		Nutrient composition (g/kg DM)	
Crude Protein (CP)	43	Crude Protein (CP)	177
Crude Fat (EE)	20	Crude Fat (EE)	8
Crude Ash (CA)	71.5	Crude Ash (CA)	290
Crude Fiber (CF)	320	pH	4.06
NDF	675	Water Soluble Carbohydrate (WSC)	420
ADF	428		

Table 2. Various treatments of wheat straw and other ingredients for ensiling.

Treatment Groups	Straw (g)	Additives (g)
Control (W)	600	--
W + Urea + Molasses (WUM)	600	Urea: 8.326 Molasses: 75.6
W + Water (WH)	600	Water: 787.50
W + Water + Urea+ Molasses (WHUM))	600	Water: 857.50 Urea: 8.326 Molasses: 75.6
W + Silage effluent (WS)	600	Silage effluent: 1125
W+50% Silage effluent + 50% water + Urea + Molasses (WSHUM)	600	Silage effluent 577.50 Water 408.7 Molasses: 37 Urea: 4.12

Table 3. Physical (color, smell and moldiness) characters and microbial composition of silages under various treatment after 45 days of fermentation.

Treatment[1] Groups	Color	Smell	Observation	Total	LAB[2]	Coliform bacteria	Mold Yeast
WUM	Yellow	Slight vinegar		$4.1*10^8$	$4.1*10^4$	ND	$1.1*10^5$
WH	Blackish brown	Putrefying	8-10 cm moldiness	$2*10^{10}$	$4.1*10^6$	$^*>10^8$	$4.6*10^7$
WHUM	Light yellow	Vinegar	2-3 cm moldiness	$8.4*10^8$	$6.8*10^9$	ND	$7.6*10^7$
WS	Brownish yellow	Typical fruity		$5.9*10^8$	$2.5*10^8$	ND	ND
WSHUM	Brownish Yellow	Typical fruity		$5.9*10^9$	$6.3*10^8$	ND	ND

[1]In treatments: WUM=Wheat straw-urea-molasses, WH= Wheat straw-water, WHUM= Wheat straw-water- urea+ molasses, WS= Wheat straw-silage effluent WSHUM = Wheat straw-50% silage effluent-50% water-urea- molasses
[2]LAB= Lactic acid bacteria, ND= Not detected, *= Uncountable

Table 4. Chemical composition of various treatment groups after 45 days of fermentation.

Item	Treatment[1]						
Chemical Composition	**Control (W)**	**WUM**	**WH**	**WHUM**	**WS**	**WSHUM**	**SEM**
				g/kg			
DM	940.56	941.87	946.07	938.58	933.3	940.04	1.78
Ash	100.36	99.79	102.45	102.92	141.08	120.95	6.97
CP	48.66	85.12	55.08	79	76.32	98.8	3
CF	323.75	325.15	372.15	355.6	285.05	305.5	3.9
NDF	675.35	682.3	722.85	704.4	570.75	619.15	5.1
ADF	427.55	437.05	484	450.8	366.15	398.4	6.03

[†] In treatments: WUM=Wheat straw-urea-molasses, WH= Wheat straw-water, WHUM= Wheat straw-water- urea+ molasses, WS= Wheat straw-silage effluent WSHUM = Wheat straw-50% silage effluent-50% water-urea- molasses
SEM= Standard error of mean

Table 5. ***In situ*** **rumen digestion kinetics and effective degradability of nutrients (g/kg) in control and treated feed groups.**

Item[1]	Treatments							
	Control (W)	WUM	WH	WHUM	WS	WSHUM	SEM[2]	P values
Dry matter (DM)								
A	0.104^{d}	0.119^{c}	0.066^{e}	0.142^{b}	0.162^{a}	0.167^{a}	0.003	≤ 0.001
B	0.439^{c}	0.403^{cd}	0.377^{d}	0.504^{b}	0.581^{a}	0.611^{a}	0.017	≤ 0.001
C	0.015^{e}	0.054^{a}	0.027^{d}	0.04^{b}	0.034^{c}	0.029^{cd}	0.002	≤ 0.001
a+b	0.543^{c}	0.521^{c}	0.443^{d}	0.646^{b}	0.743^{a}	0.778^{a}	0.018	≤ 0.001
ED[2]	0.289^{d}	0.41^{c}	0.277^{d}	0.476^{b}	0.529^{a}	0.53^{a}	0.008	≤ 0.001
ED[5]	0.203^{d}	0.326^{c}	0.194^{d}	0.365^{b}	0.398^{a}	0.393^{a}	0.006	≤ 0.001
ED[8]	0.172^{d}	0.279^{c}	0.158^{e}	0.309^{b}	0.336^{a}	0.331^{a}	0.005	≤ 0.001
Organic matter (OM)								
A	0.109^{b}	0.077^{c}	0.064^{d}	0.108^{b}	0.113^{b}	0.177^{a}	0.004	≤ 0.001
B	0.36^{c}	0.596^{ab}	0.357^{c}	0.567^{b}	0.647^{a}	0.608^{ab}	0.025	≤ 0.001
C	0.04^{a}	0.016^{c}	0.029^{b}	0.02^{c}	0.02^{c}	0.018^{c}	0.003	≤ 0.001
a+b	0.469^{c}	0.673^{b}	0.421^{c}	0.675^{b}	0.761^{a}	0.784^{a}	0.026	≤ 0.001
ED[2]	0.341^{d}	0.331^{d}	0.25^{e}	0.389^{c}	0.436^{b}	0.466^{a}	0.006	≤ 0.001
ED[5]	0.262^{c}	0.215^{d}	0.177^{e}	0.269^{c}	0.299^{b}	0.339^{a}	0.005	≤ 0.001
ED[8]	0.224^{c}	0.172^{d}	0.146^{e}	0.221^{c}	0.243^{b}	0.289^{a}	0.004	≤ 0.001
Crude Protein (CP)								
A	0.076^{c}	0.176^{b}	0.069^{c}	0.369^{a}	0.387^{a}	0.381^{a}	0.006	≤ 0.001
B	0.403^{ab}	0.371^{bc}	0.365^{d}	0.302^{d}	0.434^{a}	0.384^{bc}	0.012	≤ 0.001
C	0.02^{e}	0.102^{a}	0.056^{b}	0.035^{c}	0.036^{c}	0.058^{b}	0.01	≤ 0.001
a+b	0.478^{e}	0.547^{d}	0.434^{f}	0.671^{c}	0.821^{a}	0.765^{b}	0.013	≤ 0.001
ED[2]	0.297^{d}	0.449^{b}	0.315^{d}	0.401^{b}	0.538^{a}	0.547^{a}	0.006	≤ 0.001
ED[5]	0.214^{e}	0.383^{b}	0.243^{d}	0.31^{c}	0.406^{b}	0.44^{a}	0.005	≤ 0.001
ED[8]	0.181^{d}	0.339^{b}	0.204^{d}	0.267^{c}	0.343^{b}	0.38^{a}	0.004	≤ 0.001
Acid Detergent Fiber (ADF)								
A	0.081^{d}	0.117^{c}	0.077^{d}	0.163^{b}	0.17^{ab}	0.178^{a}	0.003	≤ 0.001
B	0.29^{d}	0.435^{b}	0.293^{d}	0.437^{b}	0.496^{a}	0.385^{c}	0.012	≤ 0.001
C	0.077^{a}	0.031^{cd}	0.038^{cb}	0.025^{d}	0.046^{b}	0.04^{b}	00.00	≤ 0.001
a+b	0.371^{d}	0.553^{c}	0.371^{d}	0.6^{b}	0.665^{a}	0.563^{c}	0.012	≤ 0.001
ED[2]	0.309^{e}	0.382^{d}	0.259^{f}	0.405^{c}	0.515^{a}	0.433^{b}	0.005	≤ 0.001
ED[5]	0.255^{e}	0.284^{d}	0.196^{f}	0.308^{c}	0.407^{a}	0.348^{b}	0.003	≤ 0.001
ED[8]	0.221^{e}	0.239^{d}	0.165^{f}	0.267^{c}	0.351^{a}	0.305^{b}	0.003	≤ 0.001
Neutral Detergent Fiber (NDF)								
A	0.089^{d}	0.077^{e}	0.041^{f}	0.126^{c}	0.137^{b}	0.168^{a}	0.003	≤ 0.001
B	0.325^{cd}	0.31^{d}	0.236^{e}	0.36^{c}	0.444^{b}	0.596^{a}	0.013	≤ 0.001
C	0.014^{c}	0.052^{a}	0.05^{a}	0.047^{a}	0.03^{b}	0.014^{c}	0.00	≤ 0.001
a+b	0.414^{d}	0.387^{d}	0.276^{e}	0.486^{c}	0.581^{b}	0.664^{a}	0.015	≤ 0.001
ED[2]	0.214^{d}	0.299^{c}	0.208^{d}	0.378^{b}	0.406^{a}	0.408^{a}	0.006	≤ 0.001
ED[5]	0.156^{c}	0.234^{b}	0.158^{c}	0.3^{a}	0.306^{a}	0.295^{a}	0.004	≤ 0.001
ED[8]	0.134^{c}	0.198^{b}	0.131^{c}	0.259^{a}	0.26^{a}	0.259^{a}	0.004	≤ 0.001

Means sharing same row with different superscripts (a-f) are significantly different (P ≤ 0.05).

[1]In items: a, the quickly disappeared fractions after washing bags with running water; b, slowly degraded fractions; c, degradation rate of b per hour.

ED[2], ED[5] and ED[8]= Effective degradability, calculated by taking three different passage rates (0.02/h, 0.05/h and 0.08/h) respectively.

[2]SEM= Standard error of mean.

Conclusions: Waste material silage effluent is rich in nutrient and can be used as an inoculant, not only to improve fermentation quality of wheat straw but also to increase its degradation in rumen. Further experiments are required to clarify whether the addition of silage effluent can preserve the ensilage from undesired pathogen i.e. yeast, coliform, clostridia and fungal growth or not.

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