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DETERMINATION *IN VITRO* GAS PRODUCTION AND METHANE EMISSION VALUES OF WHEAT STRAW TREATING WITH SILAGE EFFLUENT

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ABSTRACT

This study was conducted to evaluate *in vitro* gas and methane production, gas production parameters (a + b, c), organic matter digestibility (OMD), metabolic energy (ME) and net energy lactation (NE_L) of wheat straw treated with silage effluent. Groups were formed as wheat straw (control group, (W)), straw treated with urea + molasses (WUM) and silage effluent (WS), water, urea and molasses (WHUM), water (WH), 50% silage effluent +50% water + urea + molasses (WSHUM), and then except for control group, treatment groups were subjected to 45 days anaerobic fermentation.

Crude protein and crude ash contents of the straw groups treated with silage effluent were increased ($P<0.01$), and cell wall elements such as ADF and NDF of the treatment groups were decreased ($P<0.01$). In addition, OMD, ME and NE_L contents and *in vitro* gas production (IVIG) values of straw were increased by treatment with silage effluent ($P<0.01$). It was determined that the addition of urea and molasses to wheat straw did not affect the production of methane according to the control, and *in vitro* gas production parameters were improved ($P<0.01$). In conclusion, in order to improve feed value of wheat straw, it may be treated with silage effluent cauising environmental pollution and nutrient loss, and further works should be carried out to illuminate the subject.

KEYWORDS:
Wheat straw, silage effluent, *in vitro* gas produciton, methane

INTRODUCTION

Livestock sector plays a significant role in Turkey and is essential for the food security of rural population. Rations for the ruminant animals usually comprise two different types of food; forage and concentrates. The use of forage in the nutrition of ruminant animals is a necessity both in terms of digestive physiology and cost economy because it partly meets the nutrient needs of the animals and also protects them against certain metabolic diseases [1]. Silage production from green cereal and legume plants such as corn, sorghum, alfalfa and soybean has been increased at the expense of hay or grazing in the most parts of world. Corn silage is known as one of the most important livelihood for many farms interesting in livestock production [2]. In addition, the products attained from the animals feeding with the forages with high nutritive value have a high value in terms of quality, and it also has a great importance for the human life and economy of the state. Forages are attained from natural or artificial meadows and the forage plants. However, inadequacy of forage resources in both quantitatively and qualitatively is most often the limiting factor the development of livestock production in Turkey. So, many potential agricultural by-products have been used by farmers as animal feeds. Wheat straw being one of these by-products is a major agricultural residue abundantly produced throughout the world. On an average one kg wheat grains produce 1.2-1.3 kg of straw [3]. In Turkey, approximately 29.8 million tons wheat straw is being produced annually [4]. It contains lignocellulosic material including 35-37% cellulose, 25% hemicellulose, 10-15% lignin, and 5-10% mineral and other components [5,6]. 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 [7].

Previously, treatment of wheat straw with chemicals [8,9,10], fungal enzymes [11], and lactobacillus [12] 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 [13,14] The amount of effluent produced varies between 5 and 20% depending on DM of ensiled crop [15], extent of chopping and pressing [16,17], use and type of additives [18], and pressure applied on surface [19]. Disposal of effluent is major constraint in livestock production. In previously studies, silage effluent has been fed to pigs and dairy cattle [13,20], and used for soil treatment [14]. 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 [12], but its effect on digestion has not been studied.

Although *in vivo*, in *sacco* and *in vitro* techniques are used in animal feeding studies to determine the value of feed, *in vivo* techniques are the healthiest and accurate. Today, however, *in vivo* studies are less preferred because they are expensive, take a lot of time, require more labor, and cause concern for animal welfare. To eliminate these drawbacks, Tiliey and Terry [21] and Van Soest et al. [22] have developed *in vitro* techniques to determine the digestibility of feed. It is reported that this technique requires a large number of digestive tubes and workload [23].

The aim of present experiment was to measure the effects of treating wheat straw with silaje effluent and fountain water on *in vitro* gas production and its parameters (a+b, c), methane production and performance parameters such as organic matter digestibility (OMD), metabolic energy (ME) and net energy lactation (NE_L).

MATERIALS AND METHODS

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

Feed Material. 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 [14]. Before mixing, chemical 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). 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 1 mm before in vitro gas production technique.

Rumen liquor was obtained from a rumen cannulated Holstein cow before the morning feeding. Rumen cannulated Holstein bull of 400 kg and 20 months of age was housed individually on concrete floor and fed according to 1.25x maintenance [24] ration containing 55% concentrate, 20.5% dried grass, 20.5% *alfalfa* hay and 4% wheat straw. Animal was offered *ad libitum* water throughout the experiment.

***In vitro* gas production technique.** The gas production technique was used to determine the in vitro gas production quantities (IVG) and parameters of the straw groups. Approximately 200 mg straw samples (on a dry matter basis) milled through a 1 mm sieve were incubated as in vitro in rumen fluid in the calibrated glass syrings following the procedures of Menke and Steingass [27]. Digestion medium was prepared mixing 900 ml of distilled water, 0.1 ml micromineral solution, 200 ml macromineral solution and 1 ml resazurin solution. The glass syringes were prewarmed at 39 °C and 30 ml of rumen fluid buffer mixture including 10 ml rumen fluid and 20 ml buffer solution was transferred into each syringe and then they were incubated at 39 °C in a water bath to determine the amount of gas produced in tubes at the 3, 6, 12, 24, 48, 72 and 96th of incubation times [28]. The amount

TABLE 1
Chemical compositions of wheat straw and silage effluent (%/kg DM).

Wheat Straw (W)		Silage Effluent (S)	
Dry Matter	92.5	Dry Matter	12.0
Crude Protein (CP)	4.3	Crude Protein (CP)	17.7
Ether Extract (EE)	2.0	Ether Extract (EE)	0.,8
Crude Ash (CA)	7.15	Crude Ash (CA)	2.90
Crude Fiber (CF)	32.0	pH	4.06
NDF	67.5	Water Soluble Carbohydrate (WSC)	52.5
ADF	42.8		
Water Soluble Carbohydrate (WSC)	54.55		

Dry matter (DM), crude protein (CP), ether extract (EE), crude ash (CA) and WSC of silage effluent and 45-day fermented treatment groups were analyzed according to AOAC [25]. Fiber analysis (ADF, NDF and ADL) was done using Ankom Fiber Analyser 2000 (ANKOM Technology, Macedon, NY) [26].

TABLE 2
Chemical compositions of various treatment groups after 45 days of fermentation (on DM basis, %)

Groups	DM	CA	CP	CF	NDF	ADF	EE
W	90.17	9.94^{c}	4.02^{e}	29.7^{c}	66.24^{c}	41.67^{b}	2,02^{c}
WUM	91.10	9.77^{c}	7.32^{bc}	30.4^{abc}	67.30^{bc}	42.40^{b}	2,10^{b}
WH	90.62	10.01^{c}	4.46^{d}	31.2^{a}	68.28^{ab}	45.17^{a}	1,97^{c}
WHUM	90.84	9.26^{d}	7.54^{a}	30.8^{ab}	70.12^{a}	44.04^{a}	1,99^{c}
WS	90.24	11.84^{a}	7.47^{ab}	30.1^{bc}	60.03^{d}	37.21^{d}	2,15^{ab}
WSHUM	90.25	11.18^{b}	7.26^{c}	29.8^{c}	61.23^{d}	38.56^{c}	2,20^{a}
SEM	0.90	0.10	0.07	0.30	0.66	0.42	0.02
P	0.968	0.001	0.000	0.017	0.000	0.000	0.000

a-e: Means within a column with different superscripts are significantly different.
DM: Dry matter, CA; Crude ash, CP: Crude protein, CF: Crude fiber, NDF: Nötr detergent fiber, ADF: Acid detergent fiber, EE: Ether extract, W: Wheat straw, WUM: Straw + urea-molasses, WH: Straw + water, WHUM: Straw+water+urea-molasses, WS: Straw+ silage effluent, WSHUM: straw + 50% silage effluent + 50% water + urea-molasses

of gas produced was calculated by using the NEWAY package program according to the model P = a + b (1-e^{-ct}) developed by Ørskov and McDonald [29]. Infrared methane analyzer (Sensor Europa GmbH, Erkrath, Germany model) was used to determine the methane production 24 h of *in vitro* incubation [30]. The infrared methane analyzer displayed the methane as percent of total gas. Methane production as ml was calculated as follows:

Methane Production (ml)= Total gas production (ml) x Percentage of methane (%)

Organic matter digestibility (OMD,%), metabolic energy [31] and net energy lactation (NE_L) values of straw samples [27] were calculated according to the following formulas by using CP, EE and CA contents and 24 hours gas production (GP) of the straw groups.

OMD, %: 14.88+0.89 GP + 0.45 CP + 0.065 CA

ME, (MJ/kg KM) : 2.20 + 0.136GP + 0.0574CP + 0.00286 CF^2

NEL, (MJ/kg KM) : 0.101 GP + 0.051 CP + 0.112 EE

Statistical Analysis. The data were analyzed in SPSS 17.0 [32] package program and the differences among the group averages were determined by Duncan Multiple Comparison Test [33].

RESULTS AND DISCUSSION

Chemical Composition of Treatment Groups. The average values for the chemical composition of the straw groups treated with silage effluent were showed in Table 2. Nutrient content except for DM of all treatment groups after 45-day fermentation showed varied CP, ash, CF, EE, NDF and ADF values. The dry matter contents of the straw groups were determined between 90.17% and 9.10%. Crude protein value in control group was found to be lower than those of the other treatment groups. Ash contents of treatment groups containing silage effluent increased compare to control group. The high ash content of effluent treated groups may be due to the high content of silage effluent.

CP values obtained from the current study were found to be similar to those reported by Güleçyüz and Kılıç [34], but higher than the values reported by Dhali et al [35] and Stanton and Le Valley [36]. The difference between the groups in terms of ADF, NDF and CF values were to be significant (P<0.05). Silage effluent treated wheat straws had low ADF, NDF and CF values. ADF is a measure of feed digestibility and NDF is pertinent to feed consumption [37]. It has been reported that reduction of the ADF and NDF fractions may be due to hydrolysis of fibreous material induced by microorganisms during fermentation [38]. The chemical composition of the straw in the control group showed paralellism with the results reported by many previous studies [39,40,41,42]. In addition, ADF and NDF contents in urea-molessis treated groups increased in current study. The increase in ADF and NDF values of WUM and WHUM was because of fibre-N bond. Results obtained from present study were similar to the findings of Brown et al. [43].

IVG, Methane Porduction, OMS, ME and NE_L values of Treatment Groups. The amount of in vitro gas production at 3, 6, 12, 24, 48, 72 and 96 hours of straw groups is presented in Table 3. The differences between the gas production values of the straw groups allowed to incubate in rumen were significant in all incubation times (P<0.01). Addition of urea + molasses into wheat straw and treatment of that one with silage effluent increased

TABLE 3
***In vitro* gas production values (ml / 200 mg KM) and parameters**

Grops	3	6	12	24	48	72	96	a+b	c	RSD
W	3.50^{bc}	6.50^{b}	9.50^{b}	21.50^{b}	37.44^{b}	41.25^{b}	45.13^{b}	49.89	0.026^{abc}	2.38
WUM	5.25^{a}	7.75^{a}	11.50^{a}	25.25^{a}	37.75^{b}	41.75^{b}	48.00^{a}	51.64	0.026^{abc}	2.06
WH	2.63^{c}	3.94^{c}	6.00^{c}	17.44^{c}	34.75^{b}	40.00^{b}	41.75^{c}	49.27	0.023^{bc}	3.17
WHUM	4.25^{b}	5.75^{b}	9.00^{b}	20.32^{b}	35.00^{b}	41.00^{b}	45.50^{b}	57.80	0.020^{c}	1.98
WS	5.50^{a}	8.88^{a}	12.50^{a}	26.44^{a}	42.00^{a}	44.75^{a}	47.75^{a}	51.20	0.031^{a}	2.29
WSHUM	5.50^{a}	8.50^{a}	12.50^{a}	25.00^{a}	37.75^{b}	42.00^{b}	45.75^{b}	49.72	0.027^{ab}	1.61
SEM	0.30	0.39	0.61	0.63	1.25	0.66	0.57	2.69	0.002	-
P	0.000	0.000	0.000	0.000	0.008	0.002	0.000	0.273	0.023	-

[a- c:] Means within a column with different superscripts are significantly different.
DM: Dry matter, CA; Crude ash, CP: Crude protein, CF: Crude fiber, NDF: Nötr detergent fiber, ADF: Acid detergent fiber, EE: Ether extract, W: Wheat straw, WUM: Straw + urea-molasses, WH: Straw + water, WHUM: Straw+water+urea-molasses, WS: Straw+ silage effluent, WSHUM: straw + 50% silage effluent + 50% water + urea-molasses.
a+b: potential gas production (ml); c: fractional rate of gas production (ml/h); RSD: Residual standard deviation

the amount of gas production during all incubation periods (P <0.01). In 24 hour incubation time, the highest gas production values, 26.44, 25.25 and 25.00 ml/200 mg KM which were taken into account in calculation of ME, NEL and OMD, were obtained from WS, WUM and WSHUM groups, respectively. There was no significant difference among the groups in terms of a + b values indicating the amount of potential gas production (P> 0.05).

The residual standard deviation value (RSD) is used to express the reliability of the test in in vitro gas production technique studies. It is reported that if this value is greater than 4.0 the data obtained from this technique are low reliability. Therefore, data and should not be taken into consideration and study should be repeated [44]. The results obtained from the present study showed that RSD values are within the reliable limits.

IVG produced during 24 hours of incubation, ME, NE_L, OMD and methane production values of wheat straw groups examined in the study are given in Table 4. The treatment with silage effluent and urea + molasses of wheat straw increased ME, NE_L, IVG and OMD values (P<0.01). This is due to the fact that the silage effluent, which is acidic and contains lactic acid bacteria, damaged via fermentation, disrupting the structures of cell wall elements which are difficult to digest in wheat straw. Similarly, Mehra et al. [45] stated that wheat straw treated with acetic acid and ammonia increased organic matter degradability. On the other hand, it is reported that there is a negative relationship between ADF, NDF contents of feed samples and *in vitro* OMD values. The reduction of OMD values in the straw group treated with water may be attributed to the negative impact of aerobic and pathogenic bacterias during fermentation prior to the *in vitro* technique. In terms of methane production values, silage effluent treated groups (WS, WH) were similar to control, whereas urea + molasses treated group (WUM) showed a significant decrease (P<0.05).

As a result, silage effluent increased the feed value of wheat straw by improving the performance parameters (ME, NE_L and OMD) but did not have any negative effect on methane production. In order

TABLE 4
Average values of IVG, OMD, ME, NEL and methane production of experimental groups

Groups	IVG	ME	NE_L	OMD (%)	Methane (ml)	Methane (%)
W	21.50^{b}	5.14^{b}	2.36^{b}	34.46^{b}	3.86^{b}	17.99^{a}
WUM	25.25^{a}	5.67^{a}	2.77^{a}	37.82^{a}	3.98^{b}	15.74^{b}
WH	17.44^{c}	4.59^{c}	1.99^{c}	31.06^{c}	2.74^{c}	15.69^{b}
WHUM	20.31^{b}	5.00^{b}	2.39^{b}	33.62^{b}	3.67^{b}	18.11^{a}
WS	26.44^{a}	5.83^{a}	2.72^{a}	38.97^{a}	4.67^{a}	17.67^{a}
WSHUM	25.00^{a}	5.64^{a}	2.67^{a}	37.70^{a}	4.56^{a}	18.22^{a}
SEM	0.63	0.09	0.05	0.53	0.11	0.29
P	0.000	0.000	0.000	0.000	0.000	0.000

[a-c:] Means within a column with different superscripts are significantly different.
IVG: In vitro gas production (ml), ME: Metabolizable energy (MJ/kg KM), NE_L: Net energy lactation (MJ/kg KM); OMD: Orgaic matter digestibility; W: Wheat straw, WUM: Straw + urea-molasses, WH: Straw + water, WHUM: Straw+water+urea-molasses, WS: Straw+ silage effluent, WSHUM: straw + 50% silage effluent + 50% water + urea-molasses.

to improve the feed value of the wheat straw including very low nutrient content, and to prevent the enviromental pollution and nutrient loss dou to silage effluent the treatment of wheat straw with wastel material silage effluent being rich in nutrient may be recommended for animal breeders. On the other hand, further experiments are required to clarify whether the processing wheat straw with silage effluent can improve the feed value of straw or not.

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