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Determination of the feed value, digestibility, and *in vitro* gas production of high-moisture corn grain silage

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Abstract

This study aimed to determine the feed value, digestibility, *in vitro* gas, and methane production of high-moisture corn grain silage prepared on a private farm. The nutrient content and silage quality of corn grain silage were determined. Hohenheim gas technique was used to determine the gas production of the silage, and the Daisy incubator was used to determine the *in vitro* true digestibility (IVTD). The findings revealed that high-moisture corn grain silage had a dry matter content (DM) of 66.67%, with 8.55% crude protein (CP), 3.81% crude fiber (CF), 3.19% ether extract (EE), 83.43% nitrogen-free extract (NFE), 8.20% neutral detergent fiber (NDF), and 2.38% acid detergent fiber (ADF) on a dry matter basis. The silage quality was classified as "Excellent" based on total and Flieg scoring methods. *In vitro* gas production increased during incubation, with negligible change after 48 hours. Methane production was 12.96 ml, with an *in vitro* true digestibility (IVTD) of 93.39%, metabolizable energy (ME) content of 13.42 MJ/kg DM, and potential gas production of 95.53 ml/hour. Due to its acidic structure, it has a reducing effect on enteric methane production. As a result, it is recommended that high-moisture corn grain silage, which has a higher digestibility and nutritional values than corn grain, should not be considered as a roughage source because it resembles concentrated feeds and should be given to ruminants together with another roughage source. However, its effects on animal performance should be investigated through *in vivo* studies.

Keywords: Digestibility; Feed value; High-moisture grain; *In vitro* gas production; Methane; Silage

1. Introduction

Feed expenses constitute the most considerable portion of animal production costs, around 70%. Reducing feed costs directly lowers the overall cost of animal products, which can lead to increased consumption. To achieve this, it is possible to formulate and utilize the most cost-effective rations in animal feeding, thereby reducing production costs. In this context, efforts are being made to reduce animal production costs by drying, making silage and haylage, or converting alternative roughage and concentrated feed sources into pellets in addition to their fresh use. Moreover, research has increasingly focused on utilizing alternative high-nutritional-value feed plants (such as animal beet, forage turnip, canola, guar bean, etc.) or providing roughage through germination technology in soilless agriculture applications [1,2].

Silage preparation from roughage sources is common in our country and globally. While the silage of concentrated feed sources is rare, it has been observed that corn, sorghum, etc. grains have recently been used in animal feeding by making silage, and scientific studies have rarely been carried out. Although not widespread, studies have been conducted on using high-moisture corn grain silage in different animal husbandry branches, from chicks to rabbits, horses, pigs, sheep, goats, and cattle. High-moisture corn grain silages are also made using the known traditional silage-making principles (going through the same stages as forage silages). Corn grain silage is a feed source with a moisture content of 28-35%,

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which is desired to be usable by animals and has a high energy value. However, since separating corn grains from cobs is laborious, making corn grain silage is more laborious [3].

Using rations with high-moisture corn grain silage, which is rich in starch, positively affects methane emissions, milk yield, and composition. [4]. It is known that the best silage fermentation occurs between 35-40% moisture level [5]. It is known that when the stages of silage production in high-moisture corn grain are carefully and successfully implemented, the starch digestibility for animals will be enhanced. This study evaluated the feed value, digestibility, *in vitro* gas production, and methane emissions of high-moisture corn grain silage.

2. Material and methods

2.1. Silage and rumen fluid material

Corn grain silage was supplied from a livestock farm in Kütahya Province, Turkiye. Silage samples were kept in vacuum packages until analysis. The rumen fluid used in *in vitro* studies (gas production technique and *in vitro* true digestibility) was taken from the rumen of two healthy male Anatolian buffalo, 2 years old, 400-450 kg live weight, which had just been slaughtered in the slaughterhouse and had completed rumen development. The pH value of the rumen fluid used was measured as 6.06.

2.2. Silage quality parameters

To determine the pH value of the corn grain silage, 25 g of wet sample was mixed in 100 ml of distilled water for ten minutes and then read with a digital pH meter (HANNA INSTRUMENTS 1332 model) in three replicates. The quality class of the corn grain silage was determined using sensory evaluation (odor, structure, color, and total score) and Flieg score (FS) as the following formula [6].

$$\text{Flieg score (FS)} = 220 + (2 \times \text{DM \%} - 15) - 40 \times \text{pH}$$

A silage's required pH value (RpH) is related to DM content. In other words, each silage should have a pH value, which is determined according to its DM content. The required pH value was determined by using the following formula [7]:

$$\text{Required pH (RpH)} = 0.00359 \times \text{DM (g/kg)} + 3.44$$

2.3. Chemical analysis

The corn grain silage used in the experiment was dried in a forced-air oven at 65 °C for 48 hours. Then, dried silages were milled in a hammer mill through a 1 mm sieve for chemical analysis and *in vitro* studies. Dry matter, CP, and ash analyses were determined as reported by the AOAC [8] procedure. Kjeldahl N and CP were calculated by multiplying N by 6.25. The CF, NDF, ADF, and acid detergent lignin (ADL) analyses were performed according to the method of Van Soest et al. [9] using an ANKOM^{2000} semi-automated fiber analyzer. Ether extract analysis was determined using the ANKOM^{XT15} Extraction System as specified by AOAC [8]. Organic matter (OM), cellulose (CEL), and hemicellulose (HCEL) values were determined by calculation. All chemical analyses were carried out in three replicates.

2.4. *In Vitro* Gas Production Technique

The Hohenheim gas test was used to determine the amount of *in vitro* gas production [10, 11, 12]. Gas production was measured at 3, 6, 9, 12, 24, 48, 72, and 96 hours of incubation by weighing approximately 250 mg of air-dry material from the corn grain silage samples. At the end of the experiment, the gas production values were standardized using the blank (rumen fluid + buffer mixture without sample) and standard alfalfa hay. The gas production (GP) amounts were determined using the following formula:

$$\text{GP (ml/200mg DM, 24 h)} = [(V_{24} - V_0 - GP_0) \times 200 \times (Fk + Fc) / 2] / SW$$

Where,
V_0: position of the piston at the beginning of incubation, ml;
V_{24}: position of the piston after 24 hours of incubation, ml;
GP_0: Average gas production of unsampled rumen fluid after 24 hours of incubation, ml;
SW: sample weight in mg DM.

Gas production parameters were calculated using the PC package program NEWAY according to the model reported by Ørskov and McDonald [13].

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

Where
a: amount of gas consisting of the immediately soluble fraction (ml),
b: the amount of gas formed depending on time (ml),
c: gas production rate, (ml/h), a+b: total gas production (ml),
t: incubation period (hours) and

y: "t" represents the gas production at the time.

Organic matter digestibility (OMD) was calculated from the amount of gas production, CP, and ash at 24 hours using the formula [10]:

$$OMD\ (\%) = 14.88 + 0.8893\ GP + 0.448\ CP + 0.651\ Ash$$

Metabolizable energy (ME) and net energy lactation (NE_L) contents were calculated based on the GP, CP, EE, and ash content of the silage samples using the following equations [10, 12]:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136\ GP + 0.0574\ CP + 0.002859\ EE^2$$

$$NE_L\ (MJ/kg\ DM) = 0.101\ GP + 0.051\ CP + 0.112\ EE$$

2.5. Determination of Methane Production

An infrared methane analyzer (Sensor Europa GmbH, Erkrath, Germany model) was used to determine the methane production of the silages [14]. After reading the 24-hour gas production in the *in vitro* gas production technique, the amount of methane in the gases remaining in the injectors was measured and calculated as follows:

$$\text{Methane production (ml)} = \text{total gas production (ml)} \times \text{percentage of methane (\%)}.$$

2.6. Determination of *in vitro* true digestibility

The corn grain silage's IVTD was determined with Ankom Daisy^{II} Incubator D220 [15]. Rumen fluids obtained from the animals used in the *in vitro* gas production technique were used in the study. The corn grain silage samples were placed in the Daisy incubator for 48 hours. After incubation, NDF analysis was performed on the samples remaining in the bags, and IVTD of the corn grain silage was calculated by applying the following formula:

$$IVTD,\ \% = 100 - ((W3 - (W1 \times C1)) \times 100) / W2$$

Where:
W1: weight of filter bag,
W2: sample weight,
W3: NDF amount remaining in the bag at the end of incubation,
C1: blank weight (empty bag weight after being removed from incubation and dried in the oven/original bag weight.

2.7. Data Analysis

The means and standard deviations of the data obtained in the study were determined, and the similarities and differences between the results obtained in similar studies and these results were evaluated by comparison.

3. Results and discussion

According to the results of this study, the measured pH value is lower than the expected pH value, suggesting that high-moisture corn grain, being starch-rich, does not require additives. However, due to its higher DM content, LAB inoculants should be preferred in high-moisture corn grain silage to ensure proper fermentation and prevent spoilage, as DM is typically lower in standard silages. Based on the total and Flieg scores, the corn grain silage evaluation revealed that the quality was classified as 'Excellent' according to both scoring methods.

It was determined that the DM content of corn grain silage was 66.67%, and the silage had 8.55% CP, 3.81% CF, 3.19% EE, 83.43% NFE, 8.20% NDF, and 2.38% ADF contents on a dry matter basis (Table 1). It was observed that the contents determined in the literature review and the DM, OM, CP, CF, NDF, ADF, and ash contents determined for corn grain silages in this study were similar. According to the study conducted by Eren [16], DM contents in high-moisture corn grain silage harvested at different times were reported as 65.32% – 75.31%; Canbolat [17] reported it as 70.25%, which is similar to the results obtained in this study. Additionally, Eren [16] determined that OM contents are 98.35% – 98.44% and ash contents are 1.56% – 1.65%, which is consistent with the current study.

Table 1 Nutrient contents of the high-moisture corn grain silage, as DM %

	OM	Ash	EE	CP	CF	NFE	NDF	ADF	ADL	HCEL	CEL
Mean	98.58	1.02	3.19	8.55	3.81	83.43	8.20	2.39	0.21	5.81	2.18
StD	0.28	0.22	0.41	0.03	0.21	0.81	0.40	0.14	0.03	0.27	0.10

OM: organic matter, EE: ether extract, CP: crude protein, CF: crude fiber, NFE: nitrogen-free extracts, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, HCEL: hemicellulose, CEL: cellulose, StD: standard deviation.

In the evaluation conducted with five experts experienced in silages, the quality was classified as excellent based on the total and Flieg scores (Table 2). Eren [16] reported the pH value of corn grain silage to be between 6.39 and 6.47, while the pH value of 4.68 observed in the current study is similar to that of 4.74 reported by Canbolat [17]. Hoffman et al. [18] also reported a pH value of 6.02 for the same silage. Kung et al. [19] reported the pH value as 5.45 – 5.80. This observed situation may be due to differences in fermentation conditions.

Table 2 Quality classes of the high-moisture corn grain silage concerning sensory scoring and Flieg scores

	DM %	MpH	RpH	Odor	Structure	Color	TS	QTS	FS	QFS
Mean	66.67	4.68	5.83	12	3.7	2.0	17.7	Excellent	166.14	Excellent
StD	2.64	0.38	0.92	1.65	0.63	0.07	0.89	-	5.04	-

DM: dry matter, MpH: measured pH value, RpH: required pH value, TS: total score, QTS: quality according to the total score, FS: Flieg score, QFS: quality according to Flieg score, StD: standard deviation.

In vitro gas production of high-moisture corn silage increases during the incubation period; however, the increase after 48 hours is almost negligible compared to other incubation periods. The fact that the pH value measured after the end of incubations (96 hours) is 6.50 and the inoculum does not become alkaline is considered a sign that the buffer is not depleted. In the study by Eren [16], *in vitro* gas production of high-moisture corn silage ensiled with different DM and additives increased depending on the increase in the incubation period. The *in vitro* gas production values ranged from 81.07 to 89.23 ml/200 mg DM at the 96th hour across the experimental groups, consistent with the current study's findings.

The highest *in vitro* gas production was determined as 95.53 ml/200 mg DM, which is partially consistent with the value reported by Eren [16] (89.23 ml/200 mg DM). Upon evaluating the silages for 24-hour gas production, it was found that the gas production of 77.38 ml/200 mg DM aligned with the range of 71.40 – 77.63 ml/200 mg DM reported by Eren [16] for corn grain silages harvested at different stages. Additionally, Canbolat [17] reported a 24-hour gas production of 66.63 ml/200 mg DM.

Table 3 *In vitro* gas production amounts (ml/200 mg DM) and pH values after 96 h incubation of the high-moisture corn grain silage

	Incubation time (hours)								pH*
	3	6	9	12	24	48	72	96	
Mean	15.11	35.12	51.95	62.54	77.38	92.35	95.40	95.53	6.50
StD	0.99	1.48	2.31	1.38	3.56	4.63	5.12	5.12	0.01

StD: standard deviation. * Measured pH values after 96 hours of incubation.

Table 4 shows that the methane production of the corn grain silage was 12.96 ml, the IVTD was 93.39%, the ME content was 13.42 MJ/kg DM, and the potential gas production was 95.53 ml/hour. Eren [16] reported that the IVTD of corn grain silage ranged from 85.89% to 89.00%, while the ME contents varied between 13.43 and 13.87 MJ/kg DM, and OMD ranged from 91.22% to 94.25%. These values were found to be consistent with the results of the current study. In contrast, Canbolat [18] reported ME content as 12.49 MJ/kg DM and OMD as 81.75%. The higher OMD values in this study are likely due to the high digestible starch content of high-moisture corn grain silage.

In this study, the IVTD of silage was determined as 93.39%, which is higher than the IVTD values (85.57% – 89.47%) reported by Eren [16] for high-moisture corn grain silage with varying DM contents. The observed differences in digestibility and silage fermentation are likely responsible for the variation in the results. It has been observed that high-moisture corn silage provides better health conditions and higher quality activity in dairy cows and increases nutrient intake. It is reported that this situation is due to the high energy and highly digestible starch. It is known that the starch in the structure of high-moisture corn silage is an important energy source in ruminant feeding; it is used to improve rumen fermentation, optimize the digestion of structural carbohydrates, and increase protein flow to the small intestine. While some studies suggest that feeding 8-10 kg of high-moisture corn grain silage to calves and 15-20 kg to beef and dairy cattle poses no harm, other literature indicates that feeding amounts above 8-10 kg/day may lead to health issues, primarily acidosis [20].

Table 4 Methane production, digestibilities (IVTD, OMD), energy values (ME, NE_L), and *in vitro* gas production kinetics (a, c) of the high-moisture corn grain silage

	Methan (ml)	**IVTD** (%)	**OMD** (%)	**ME** (MJ/kg DM)	**NE_L** (MJ/kg DM)	**b** (ml)	**c** (ml/h)
Mean	12.96	93.39	88.19	13.42	8.60	95.53	0.05
StD	1.14	0.80	3.16	0.48	0.36	5.12	0.00

IVTD: *in vitro* true digestibility, OMD: organic matter digestibility, ME: metabolizable energy, NEL: net energy lactation, b: potential gas production, c: the gas production rate constant for the insoluble fraction, StD: standard deviation.

As a result, making corn grain silage is the best way to utilize corn. In this way, the animal benefits more from corn in silage production; its digestibility is higher, reflected in the animal's productivity. It has an energy and protein content close to dry corn. Since the moisture content is reached early in high-moisture corn grain silage, the field is plowed early, providing the second crop planting opportunity. It is less likely to be affected by natural disasters such as frost and flood. High-moisture corn grain silage can be stored long and fed to animals yearly. However, if it is not ensiled under suitable conditions, the reproduction of mold, fungus, and toxins is much easier and higher, and the quality will be decreased. Therefore, the moisture level must be adjusted appropriately (between 24% and 35%). If added to the ration above a specific limit, there is a risk of acidosis. Absorption from the rumen and intestines is fast, and starch utilization is higher.

4. Conclusion

High-moisture corn grain silage, which is still in the widespread stage worldwide, should be preferred, especially in regions where there is a problem with drying corn grain when the field is wanted to be emptied early for the second crop planting, when it is wanted to benefit from the advantages of silage production and when it is wanted to increase the digestibility of corn grain. As a result, there are not enough studies on using high-moisture corn grain silage in animals, which have higher digestibility and nutritional values than corn grain. Although silage, it should not be evaluated as a sole roughage source because its content is similar to concentrated feeds. Another roughage source should be included in the ration, and a maximum of 10 kg should be given to adult cattle per day against the risk of acidosis. It can also be used in other ruminants, monogastric animals, and poultry.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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