



## IN VITRO DIGESTIBILITY OF DIFFERENT HYDROPONIC GRAIN SOURCES AND USE IN RUMINANT FEEDING

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**Abstract:** Forage crops are an essential nutrient source in animal nutrition for meeting nutrient requirements and stimulating appetite as green forage. However, providing fresh forage to animals throughout the year is challenging or even impossible in our country. This situation, due to limited cultivation areas and insufficient yield per unit area, prevents the complete fulfillment of roughage and concentrate feed requirements of farm animals in terms of quality and quantity. This leads to productivity losses in animal production and increased feed costs. Therefore, various studies are currently being conducted to ensure the supply of green forage throughout the year. For this purpose, in this study, the nutritional composition (dry matter, ASH, crude protein, crude fat, crude fiber, NDF, ADF), in vitro digestibility parameters, and methane production levels of hydroponically produced forms of barley, wheat, corn, oat, and sorghum grains cultivated in the Niğde region were determined. Raw, room temperature-dried, and oven-dried forms of hydroponic grains were compared. Nutrient composition, energy values, in vitro gas production, pH, ammonia concentration, and methane production were evaluated to determine their suitability for ruminant nutrition. The findings revealed that although hydroponic grains provided high crude protein content (15.96-16.04%), organic matter digestibility (47.92-82.80%) and energy values (ME ranging from 7.59-12.59 MJ/kg DM) showed variability. Overall, the findings indicate that the suitability of hydroponically produced grains for ruminant nutrition depends on grain species and post-harvest processing method.

**Keywords:** Forage crops, Soilless farming, Hydroponic production, Ruminant feeding, *In vitro* gas production, Methane

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Received: April 08, 2026

Accepted: June 16, 2026

Published: July 15, 2026

Cite as: Boğa, M., & Kiliç Özel, H. N. (2026). *In vitro* digestibility of different hydroponic grain sources and use in ruminant feeding. *Black Sea Journal of Agriculture*, 9(4), 575-583.

### 1. Introduction

Sustainable livestock production depends on the continuous availability and quality of forage crops. Forage cultivation is primarily shaped by the size of agricultural land, soil characteristics, and regional climate (Boğa et al., 2023). Traditional soil-based systems are particularly vulnerable to climatic variability, which in turn drives up production costs (Boğa et al., 2023). Water use in agriculture is the single most limiting factor for economic efficiency, especially in arid and semi-arid regions. Increasing challenges including groundwater depletion, high evapotranspiration rates, and poor water retention in soils place growing pressure on conventional irrigation (Al-Karaki and Al-Hashimi, 2011). Hydroponic systems offer a practical alternative, combining a hygienic growing environment, pest-free conditions, high yield potential, year-round production, and minimal water consumption (Al-Karaki, 2011; Boğa et al., 2023). Recent reviews have highlighted the increasing importance of hydroponic forage systems as sustainable alternatives for livestock production under conditions of water scarcity and limited arable land. Hydroponically produced

fodders can provide year-round forage availability while improving resource-use efficiency; however, their nutritional composition, digestibility, and feeding value vary considerably according to grain species, cultivation conditions, and harvest stage. Therefore, further evaluations of different hydroponic grain sources are required to optimize their utilization in ruminant nutrition (Pastorelli et al., 2024; Vastolo and Cutrignelli, 2025).

The importance of soilless agriculture has grown considerably in recent years, driven by shrinking arable land, low yield per unit area, and deteriorating soil quality. In small-scale livestock enterprises, the constraints are compounded further: insufficient land for forage cultivation, limited water supply, difficulties in sourcing quality seed, high labour demands, fertiliser requirements, and the inherently long growth period of conventional forage crops (45–60 days) all restrict roughage availability (Naik et al., 2015). These limitations have pushed hydroponic production to the forefront as an alternative feed supply strategy (Boğa et al., 2023). Hydroponic production is based on growing plants in nutrient solutions without soil. Unlike



conventional systems, where micronutrient supply is often neglected, hydroponic systems deliver all essential elements directly to the root zone, contributing meaningfully to yield improvement (Gül, 2008; Hazar and Bakır, 2013). In ruminant nutrition specifically, hydroponic fodder is produced within six to seven days, without soil, in nutrient-enriched solutions though plain tap water has also been reported as a viable medium (Naik et al., 2015). Studies have reported improvements in digestibility, nutrient utilization, and animal performance following the inclusion of hydroponic fodder in ruminant diets (Naik et al., 2015; Raeisi et al., 2018). Improvements in crude protein, fibre, vitamin, and mineral content further raise the feed quality of germinated grains (Lorenz and D'Appolonia, 1980). The absence of toxic agrochemicals in this production system represents an additional advantage for both animal and human health (Kılıç, 2016; Boğa et al., 2023).

The continuity of animal production, reproductive performance, and milk yield depends substantially on access to quality green forage; its absence adversely affects all of these parameters (Naik et al., 2014). Given that feed costs represent approximately 75% of total expenditure in milk production with green roughage accounting for around 30% of the roughage fraction (Indira et al., 2020) optimising the price-quality balance of hydroponic feed is of direct practical importance.

Hydroponic fodders have been investigated in numerous studies; however, reported results vary considerably according to grain species, cultivation conditions, harvest stage, and post-harvest processing methods. Such variability makes direct comparisons among studies difficult and highlights the importance of evaluating different hydroponic grain sources under comparable experimental conditions. Therefore, the aim of this study was to evaluate the potential use of hydroponically produced barley, wheat, corn, oat, and sorghum in ruminant nutrition by determining their nutritional composition (dry matter, ash, crude protein, crude fat, crude fibre, NDF, ADF), *in vitro* digestibility parameters, and methane production levels. Raw, room temperature-dried, and oven-dried forms of each grain were compared in terms of nutrient content, energy values, *in vitro* gas production, pH, ammonia, and methane output. In addition, the study simultaneously examined digestibility, energy values, rumen fermentation characteristics, and methane production across all grain sources, providing an integrated assessment of their potential feeding value for ruminants.

## 2. Materials and Methods

### 2.1. Feed and Animal Material

Barley, wheat, corn, oat, and sorghum grains produced in the Niğde region were purchased from a local supplier after harvest. The grains were used for hydroponic cultivation and subsequent laboratory analyses. Rumen fluid used as the inoculum was collected from freshly slaughtered cattle at a local abattoir.

### 2.2. Hydroponic Cultivation

Hydroponic cultivation was conducted in the laboratory of Niğde Ömer Halisdemir University, Bor Vocational School. Grains were germinated in individual trays at 15–35 °C with relative humidity maintained at 80–90% by scheduled water application throughout the day. Germination was completed within six to seven days. Hydroponic cultivation was carried out in perforated plastic trays. The hydroponic fodders were produced in perforated plastic crates with dimensions of 30 × 40 × 14 cm (W × L × H). No nutrient solution was used during the production period, and irrigation was performed exclusively with tap water. On the first day of germination, water was applied by spraying at approximately one-hour intervals to maintain adequate moisture. During the subsequent growth period, irrigation was carried out three times daily (morning, noon, and evening). The fodders were harvested after 6–7 days of growth and subsequently subjected to the designated drying treatments and laboratory analyses. After the germination period (6–7 days), the harvested hydroponic fodders were divided into three treatment groups. The first group was analyzed immediately after harvest and was referred to as the “raw” treatment throughout the manuscript. Therefore, the term “raw” does not refer to ungerminated grains but to fresh hydroponically produced fodder analyzed without any drying process. The second group was dried at room temperature, whereas the third group was dried in a forced-air oven prior to analysis.

### 2.3. Chemical Analyses

Dry matter (DM), crude protein (CP), crude fat (EE), crude fibre (CF), and crude ash (ASH) were determined according to AOAC (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were analysed following Van Soest et al. (1991); nitrogen-free extractives (NFE) were calculated by difference. All analyses were performed in five replicates.

### 2.4. *In Vitro* Gas Production and Energy Estimation

The *in vitro* gas production technique was applied using 100 mL glass syringes as described by Menke et al. (1979), Menke and Steingass (1988), and Blümmel and Ørskov (1993). Gas volumes were recorded at 0–3, 6, 9, 12, 24, 48, 72, and 96 hours of incubation. Ammonia concentration was determined from rumen fluid collected after 96 hours. Organic matter digestibility (OMD), metabolizable energy (ME), and net energy lactation (NEL) were estimated using the following equations 1–3:

$$\text{OMD (\%)} = 14.88 + 0.889 \text{ GP} + 0.45 \text{ CP} + 0.065 \text{ ASH} \quad (1)$$

$$\text{ME (MJ/kg DM)} = 1.06 + 0.157 \text{ GP} + 0.00884 \text{ CP} + 0.022 \text{ EE} - 0.0081 \text{ ASH} \quad (2)$$

$$\text{NEL (MJ/kg DM)} = 0.075 \text{ GP} + 0.087 \text{ CP} + 0.161 \text{ EE} + 0.056 \text{ NFE} - 2.422 \quad (3)$$

where GP: gas production at 96 h (mL/200 mg DM); CP, EE, ASH, and NFE are expressed as g/kg DM.

## 2.5. Statistical Analysis

Data was analysed according to a randomized complete block design. Duncan's multiple range test was used to compare treatment means (SPSS v.13); significance was set at  $P < 0.05$  (\*) and  $P < 0.01$  (\*\*). All chemical analyses and *in vitro* measurements were performed in five replicates. The experimental treatments consisted of five hydroponically produced grain species (barley, wheat, corn, oat, and sorghum) evaluated under three processing conditions (raw, room temperature-dried, and oven-dried). Analysis of variance (ANOVA) was performed prior to mean separation using Duncan's multiple range test (Genç and Soysal, 2018).

## 3. Results

### 3.1. Nutritional Composition

Nutritional composition of hydroponically produced grains is presented in Table 1. Oven-dried samples consistently showed the highest DM values (corn oven:  $95.62 \pm 0.05\%$ ; sorghum oven:  $95.14 \pm 0.06\%$ ; oat oven:  $95.06 \pm 1.10\%$ ), while raw samples recorded the lowest (corn raw:  $88.99 \pm 0.06\%$ ; wheat raw:  $90.15 \pm 0.02\%$ ). ASH content peaked under oven conditions (oat oven:  $4.36 \pm 0.67\%$ ; corn oven:  $3.66 \pm 0.46\%$ ) and was lowest in wheat raw ( $1.87 \pm 0.26\%$ ) and corn room temperature-dried ( $1.78 \pm 0.24\%$ ). CP varied widely by grain and treatment, ranging from

7.15% in corn oven to 16.04% in oat oven; sorghum remained within 7–9% regardless of treatment. Oat had the highest EE across all drying methods (4.79–7.21%), while other grains ranged between 2 and 3.5%, with barley room temperature-dried the lowest ( $1.95 \pm 0.02\%$ ). CF was greatest in oat samples (15.18–15.67%) and lowest in sorghum raw ( $3.05 \pm 0.87\%$ ) and wheat raw ( $3.45 \pm 0.34\%$ ).

NDF was highest in wheat room temperature-dried ( $33.23 \pm 0.07\%$ ) and oat samples (29.69–31.62%), and lowest in corn (9.38–9.94%) and sorghum (9.71–14.88%). ADF followed the same pattern, peaking in wheat room temperature-dried ( $16.04 \pm 0.00\%$ ) and oat (14.43–14.49%), and dropping to below 3% in all corn treatments. ADL was greatest in wheat oven ( $2.42 \pm 0.05\%$ ) and oat (2.17–2.20%), and lowest in corn (0.24–0.32%).

### 3.2. Organic Matter Digestibility, Metabolizable Energy, and Net Energy Lactation

Organic matter digestibility, metabolizable energy, and net energy lactation data are presented in Table 2. Raw wheat recorded the highest OMD ( $82.80 \pm 2.58\%$ ), ME ( $12.59 \pm 0.40$  MJ/kg DM), and NEL ( $7.71 \pm 0.30$  MJ/kg DM) of all treatments, whereas raw oat consistently returned the lowest values (OMD:  $47.92 \pm 3.94\%$ ; ME:  $7.59 \pm 0.60$  MJ/kg DM; NEL:  $3.75 \pm 0.40$  MJ/kg DM).

**Table 1.** Nutrient content of hydroponically produced barley, wheat, corn, oats, and sorghum

| Hydroponically Produced        | DM. %                   | ASH. % DM               | CP. % DM                 | EE. % DM                | CF% DM                  | NDF. % DM               | ADF. % DM              | ADL. % DM              |
|--------------------------------|-------------------------|-------------------------|--------------------------|-------------------------|-------------------------|-------------------------|------------------------|------------------------|
| Barley Raw                     | $90.06 \pm 0.06^{c**}$  | $2.24 \pm 0.14^{cd**}$  | $14.53 \pm 0.14^{ab**}$  | $2.10 \pm 0.10^{bcd**}$ | $5.24 \pm 1.18^{cd**}$  | $19.91 \pm 1.43^{cd**}$ | $5.96 \pm 0.50^{de**}$ | $0.79 \pm 0.12^{cd**}$ |
| Barley Room Temperature-Dried  | $91.04 \pm 0.10^{d**}$  | $2.46 \pm 0.09^{bc**}$  | $13.97 \pm 0.26^{bc**}$  | $1.95 \pm 0.02^{cd**}$  | $11.30 \pm 0.79^{b**}$  | $28.50 \pm 0.19^{cd**}$ | $11.98 \pm 0.03^{c**}$ | $1.33 \pm 0.07^{bc**}$ |
| Barley Oven                    | $93.13 \pm 0.08^{b**}$  | $3.29 \pm 0.04^{a**}$   | $13.22 \pm 0.42^{cde**}$ | $2.14 \pm 0.04^{bcd**}$ | $11.77 \pm 0.13^{b**}$  | $28.09 \pm 0.14^{cd**}$ | $11.71 \pm 0.04^{c**}$ | $1.33 \pm 0.01^{a**}$  |
| Wheat Raw                      | $90.15 \pm 0.02^{e**}$  | $1.87 \pm 0.26^{e**}$   | $12.21 \pm 0.35^{e**}$   | $2.28 \pm 0.03^{bc**}$  | $3.45 \pm 0.34^{e**}$   | $15.04 \pm 1.02^{f**}$  | $3.57 \pm 0.04^{e**}$  | $0.73 \pm 0.04^{e**}$  |
| Wheat Room Temperature-Dried   | $92.02 \pm 0.02^{c**}$  | $2.70 \pm 0.15^{b**}$   | $15.96 \pm 0.18^{a**}$   | $3.25 \pm 0.13^{a**}$   | $14.09 \pm 3.84^{b**}$  | $33.23 \pm 0.07^{b**}$  | $16.04 \pm 0.00^{b**}$ | $1.96 \pm 0.04^{a**}$  |
| Wheat Oven                     | $93.77 \pm 0.17^{b**}$  | $2.67 \pm 0.64^{b**}$   | $13.49 \pm 0.35^{cd**}$  | $3.54 \pm 0.03^{a**}$   | $14.09 \pm 3.84^{b**}$  | $28.28 \pm 0.54^{cd**}$ | $11.63 \pm 0.05^{c**}$ | $2.42 \pm 0.05^{a**}$  |
| Corn Raw                       | $88.99 \pm 0.06^{f**}$  | $2.85 \pm 0.44^{cde**}$ | $10.71 \pm 4.58^{ef**}$  | $2.33 \pm 0.11^{bc**}$  | $3.91 \pm 0.53^{de**}$  | $9.44 \pm 0.46^{e**}$   | $2.26 \pm 0.06^{e**}$  | $0.32 \pm 0.03^{f**}$  |
| Corn Room Temperature-Dried    | $90.73 \pm 0.01^{de**}$ | $1.7 \pm 0.24^{e**}$    | $7.24 \pm 0.61^{f**}$    | $2.85 \pm 0.11^{b**}$   | $6.00 \pm 0.09^{cde**}$ | $9.38 \pm 0.04^{e**}$   | $2.60 \pm 0.02^{e**}$  | $0.24 \pm 0.00^{f**}$  |
| Corn Oven                      | $95.62 \pm 0.05^{a**}$  | $3.66 \pm 0.46^{a**}$   | $7.15 \pm 0.20^{f**}$    | $2.99 \pm 0.04^{b**}$   | $4.55 \pm 0.97^{de**}$  | $9.94 \pm 0.66^{e**}$   | $2.83 \pm 0.11^{e**}$  | $0.32 \pm 0.04^{f**}$  |
| Oat Raw                        | $90.60 \pm 0.46^{de**}$ | $3.84 \pm 0.21^{ab**}$  | $10.29 \pm 0.90^{ef**}$  | $4.79 \pm 0.33^{a**}$   | $15.37 \pm 1.39^{ab**}$ | $29.69 \pm 1.35^{c**}$  | $12.05 \pm 0.32^{c**}$ | $2.17 \pm 0.18^{bc**}$ |
| Oat Room Temperature-Dried     | $91.01 \pm 0.02^{d**}$  | $3.81 \pm 0.22^{ab**}$  | $11.78 \pm 0.13^{de**}$  | $6.43 \pm 0.16^{a**}$   | $15.18 \pm 0.72^{ab**}$ | $30.63 \pm 0.14^{c**}$  | $14.49 \pm 0.42^{b**}$ | $2.20 \pm 0.01^{bc**}$ |
| Oat Oven                       | $95.06 \pm 1.10^{a**}$  | $4.36 \pm 0.67^{a**}$   | $16.04 \pm 0.51^{a**}$   | $7.21 \pm 0.06^{a**}$   | $15.67 \pm 1.57^{ab**}$ | $31.62 \pm 0.75^{bc**}$ | $14.43 \pm 0.91^{b**}$ | $1.97 \pm 0.61^{a**}$  |
| Sorghum Raw                    | $90.51 \pm 0.02^{de**}$ | $1.90 \pm 0.36^{e**}$   | $7.36 \pm 0.33^{f**}$    | $3.16 \pm 0.08^{ab**}$  | $3.05 \pm 0.87^{e**}$   | $9.71 \pm 0.57^{e**}$   | $4.23 \pm 0.19^{f**}$  | $1.31 \pm 0.01^{e**}$  |
| Sorghum Room Temperature-Dried | $89.53 \pm 0.11^{f**}$  | $2.23 \pm 0.21^{de**}$  | $7.91 \pm 0.04^{f**}$    | $2.46 \pm 0.09^{bc**}$  | $8.29 \pm 0.63^{c**}$   | $14.23 \pm 0.70^{f**}$  | $6.48 \pm 0.17^{e**}$  | $1.75 \pm 0.07^{c**}$  |
| Sorghum Oven                   | $95.14 \pm 0.06^{a**}$  | $2.93 \pm 0.26^{bc**}$  | $8.81 \pm 0.59^{f**}$    | $2.32 \pm 0.09^{bc**}$  | $4.69 \pm 0.02^{de**}$  | $14.88 \pm 0.62^{f**}$  | $6.78 \pm 0.05^{e**}$  | $1.85 \pm 0.05^{c**}$  |
| Standard Error                 | 0.05                    | 0.05                    | 0.19                     | 0.02                    | 0.24                    | 0.11                    | 0.05                   | 0.03                   |

\* DM= dry matter, ASH= crude ash, EE= ether extract, CP= crude protein, CF= crude fiber, NDF= neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, means with different superscripts in the same column are statistically significantly different, SE= standard error,  $\pm$ = standard deviation, ( $P < 0.05$ \*,  $P < 0.01$ \*\*).

**Table 2.** Organic matter digestibility (OMD), metabolizable energy (ME), net energy lactation (NEL) contents of hydroponically produced barley, wheat, corn, oat, and sorghum

| Hydroponically Produced        | OMD (%)                    | ME (MJ/kg DM)             | NEL (MJ/kg DM)           |
|--------------------------------|----------------------------|---------------------------|--------------------------|
| Barley Raw                     | 70.61±3.73 <sup>d**</sup>  | 10.72±0.57 <sup>a**</sup> | 6.33±0.42 <sup>c**</sup> |
| Barley Room Temperature-Dried  | 61.24±1.00 <sup>f**</sup>  | 9.29±0.15 <sup>c**</sup>  | 5.27±0.11 <sup>e**</sup> |
| Barley Oven                    | 65.17±1.41 <sup>e**</sup>  | 9.89±0.21 <sup>b**</sup>  | 5.71±0.16 <sup>d**</sup> |
| Wheat Raw                      | 82.80±2.58 <sup>a**</sup>  | 12.59±0.40 <sup>d**</sup> | 7.71±0.30 <sup>a**</sup> |
| Wheat Room Temperature-Dried   | 58.16±7.79 <sup>g**</sup>  | 8.64±1.02 <sup>d**</sup>  | 4.78±0.76 <sup>f**</sup> |
| Wheat Oven                     | 66.82±2.65 <sup>e**</sup>  | 10.15±0.40 <sup>b**</sup> | 5.90±0.30 <sup>d**</sup> |
| Corn Raw                       | 69.20±10.48 <sup>d**</sup> | 10.51±1.60 <sup>a**</sup> | 5.81±1.20 <sup>d**</sup> |
| Corn Room Temperature-Dried    | 69.10±5.10 <sup>d**</sup>  | 9.89±0.40 <sup>b**</sup>  | 5.65±0.30 <sup>d**</sup> |
| Corn Oven                      | 70.64±6.60 <sup>d**</sup>  | 10.23±0.80 <sup>b**</sup> | 5.75±0.50 <sup>d**</sup> |
| Oat Raw                        | 47.92±3.94 <sup>i**</sup>  | 7.59±0.60 <sup>e**</sup>  | 3.75±0.40 <sup>g**</sup> |
| Oat Room Temperature-Dried     | 53.79±6.97 <sup>h**</sup>  | 8.83±1.00 <sup>d**</sup>  | 4.44±0.80 <sup>f**</sup> |
| Oat Oven                       | 51.83±2.32 <sup>h**</sup>  | 7.86±0.30 <sup>e**</sup>  | 4.23±0.20 <sup>f**</sup> |
| Sorghum Raw                    | 64.05±8.50 <sup>e**</sup>  | 9.72±1.20 <sup>b**</sup>  | 5.59±0.90 <sup>d**</sup> |
| Sorghum Room Temperature-Dried | 74.75±3.20 <sup>c**</sup>  | 11.37±0.40 <sup>c**</sup> | 6.79±0.30 <sup>b**</sup> |
| Sorghum Oven                   | 62.76±6.44 <sup>f**</sup>  | 9.49±1.00 <sup>c**</sup>  | 5.47±0.70 <sup>d**</sup> |
| Standard Error                 | 3.14                       | 0.44                      | 0.32                     |

OMD= organic matter digestibility, ME= metabolizable energy, NEL= net energy lactation. Means with different superscripts in the same column are statistically significant, SE= standard error, ±= standard deviation (SD), (P<0.05\*, P<0.01\*\*).

Room temperature-dried caused the greatest energy losses in wheat (OMD: 58.16±7.79%) and barley (OMD: 53.48±5.60%), while oven drying preserved comparatively higher values in both species. Corn OMD remained stable across all treatments (69.10–70.64%), though raw corn showed high within-group variation (±10.48). Notably, room temperature-dried sorghum produced higher OMD (74.75±3.20%), ME (11.37±0.40 MJ/kg DM), and NEL (6.79±0.30 MJ/kg DM) than both its raw and oven-dried counterparts.

### 3.3. In Vitro Gas Production

Gas production data are presented in Table 3.

All grains showed a time-dependent increase in gas volume, reaching maximum values at 96 h. Raw wheat produced the highest gas volume (96.05±3.58 mL/200 mg DM), while oat consistently recorded the lowest across all treatments (51.79–62.32 mL/200 mg DM at 96 h). Barley raw (81.43±3.92 mL) exceeded room temperature-dried (73.20±2.25 mL) and oven (78.52±2.47 mL) values. Corn was largely unaffected by drying treatment (75.79–77.79 mL/200 mg DM). Room temperature-dried sorghum produced notably higher gas volumes (82.95±2.87 mL) than raw (69.72±6.01 mL) and oven-dried (67.04±5.15 mL) forms.

### 3.4. Methane, Ammonia, and pH

Methane, ammonia, and pH data are presented in Table 4. Ammonia production was highest in room temperature-dried groups and lowest under oven conditions across all grains: oat room temperature-dried reached the overall maximum (61.0±1.0 mg/100 g) and sorghum oven the minimum (8.5±0.5 mg/100 g). Methane production

varied less among treatments than ammonia but differed by species. Wheat samples produced the highest methane content (23.40–23.73%) and sorghum raw the lowest (19.00±1.04%); barley and wheat consistently exceeded other grains in this regard. pH was determined primarily by grain type rather than drying method: corn was lowest (6.44–6.46), oat highest (6.87–6.93), and barley remained stable and near-neutral (6.76–6.80).

## 4. Discussion

### 4.1. Nutritional Composition

The high DM values recorded in oven-dried samples reflect the concentration effect of moisture removal, consistent with findings reported for hydroponically grown oat and barley (Hillier and Perry, 1969; Peer and Leeson, 1985). The low DM observed in raw samples is attributable to starch catabolism during germination, which reduces both organic matter and DM content a pattern well documented in the hydroponic literature, with reported DM losses ranging from 9.4 to 89.7% (Fazaeli et al., 2011; Naik et al., 2015; Boğa et al., 2023). During germination, photosynthesis begins around day five, but the activation of chloroplasts at this stage leaves insufficient time for DM accumulation (Dung et al., 2010). The increase in ASH under oven drying is consistent with the elevated mineral concentration associated with water loss.

**Table 3.** *In vitro* gas production contents of hydroponically produced barley, wheat, corn, oat, and sorghum

| Hydroponically Produced        | 0-3.hour                | 6.hour                  | 9.hour                  | 12. hour                | 24.hour                  | 48.hour                   | 72.hour                   | 96.hour                   |
|--------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|---------------------------|---------------------------|---------------------------|
| Barley Raw                     | 16.74±0.68 <sup>a</sup> | 33.49±1.35 <sup>a</sup> | 50.23±2.03 <sup>a</sup> | 52.75±2.81 <sup>a</sup> | 62.66±3.95 <sup>a</sup>  | 70.65±2.78 <sup>a</sup>   | 74.75±2.90 <sup>a</sup>   | 81.43±3.92 <sup>a</sup>   |
| Barley Room Temperature-Dried  | 12.98±0.23 <sup>b</sup> | 25.96±0.46 <sup>b</sup> | 38.93±0.68 <sup>b</sup> | 41.17±1.13 <sup>b</sup> | 52.13±0.72 <sup>b</sup>  | 61.16±1.71 <sup>b</sup>   | 66.36±1.86 <sup>b</sup>   | 73.20±2.25 <sup>b</sup>   |
| Barley Oven                    | 13.87±0.23 <sup>b</sup> | 27.73±0.46 <sup>b</sup> | 41.60±0.68 <sup>b</sup> | 45.25±1.35 <sup>b</sup> | 56.55±1.30 <sup>b</sup>  | 65.91±1.49 <sup>b</sup>   | 71.39±1.84 <sup>b</sup>   | 78.52±2.47 <sup>b</sup>   |
| Wheat Raw                      | 20.83±0.59 <sup>a</sup> | 41.67±1.18 <sup>a</sup> | 62.49±1.77 <sup>a</sup> | 65.20±1.09 <sup>a</sup> | 76.37±1.85 <sup>a*</sup> | 86.07±2.92 <sup>a**</sup> | 89.68±3.16 <sup>a**</sup> | 96.05±3.58 <sup>a**</sup> |
| Wheat Room Temperature-Dried   | 11.00±1.30 <sup>b</sup> | 21.99±2.59 <sup>b</sup> | 32.99±3.89 <sup>b</sup> | 36.72±4.03 <sup>b</sup> | 48.67±5.16 <sup>b</sup>  | 56.57±6.22 <sup>b</sup>   | 61.98±6.92 <sup>b</sup>   | 68.13±7.06 <sup>b</sup>   |
| Wheat Oven                     | 14.97±0.63 <sup>c</sup> | 29.94±1.26 <sup>c</sup> | 44.92±1.89 <sup>c</sup> | 47.86±1.75 <sup>c</sup> | 58.24±2.22 <sup>c</sup>  | 68.15±2.73 <sup>c</sup>   | 73.57±2.88 <sup>c</sup>   | 80.21±2.96 <sup>c</sup>   |
| Corn Raw                       | 14.55±1.35 <sup>a</sup> | 29.11±2.70 <sup>a</sup> | 43.66±4.04 <sup>a</sup> | 49.14±4.99 <sup>a</sup> | 61.08±7.24 <sup>a</sup>  | 68.75±7.37 <sup>a</sup>   | 71.60±7.33 <sup>a</sup>   | 77.79±7.53 <sup>a</sup>   |
| Corn Room Temperature-Dried    | 17.16±0.37 <sup>b</sup> | 34.32±0.75 <sup>b</sup> | 51.47±1.12 <sup>b</sup> | 55.63±1.05 <sup>b</sup> | 60.97±3.20 <sup>a</sup>  | 67.09±3.94 <sup>a</sup>   | 69.94±4.25 <sup>a</sup>   | 75.90±4.05 <sup>a</sup>   |
| Corn Oven                      | 16.80±0.97 <sup>b</sup> | 33.61±1.93 <sup>b</sup> | 50.41±2.90 <sup>b</sup> | 53.45±3.43 <sup>b</sup> | 62.71±4.45 <sup>a</sup>  | 67.50±4.46 <sup>a</sup>   | 69.90±4.77 <sup>a</sup>   | 75.79±4.69 <sup>a</sup>   |
| Oat Raw                        | 11.30±0.64 <sup>a</sup> | 22.60±1.28 <sup>a</sup> | 33.90±1.93 <sup>a</sup> | 32.67±2.05 <sup>a</sup> | 37.16±2.85 <sup>a</sup>  | 42.32±3.49 <sup>a</sup>   | 46.13±3.93 <sup>a</sup>   | 51.79±3.92 <sup>a</sup>   |
| Oat Room Temperature-Dried     | 12.34±1.05 <sup>a</sup> | 24.67±2.09 <sup>a</sup> | 37.01±3.14 <sup>a</sup> | 36.79±3.14 <sup>a</sup> | 43.75±4.78 <sup>a</sup>  | 49.07±5.87 <sup>a</sup>   | 53.49±6.26 <sup>a</sup>   | 59.74±6.14 <sup>a</sup>   |
| Oat Oven                       | 10.70±0.29 <sup>a</sup> | 21.40±0.59 <sup>a</sup> | 32.10±0.88 <sup>a</sup> | 32.32±0.88 <sup>a</sup> | 41.55±1.49 <sup>a</sup>  | 49.78±2.72 <sup>a</sup>   | 56.02±2.62 <sup>a</sup>   | 62.32±2.02 <sup>a</sup>   |
| Sorghum Raw                    | 11.75±0.88 <sup>a</sup> | 23.50±1.76 <sup>a</sup> | 35.25±2.64 <sup>a</sup> | 40.92±3.45 <sup>a</sup> | 55.28±5.56 <sup>a</sup>  | 60.82±5.37 <sup>a</sup>   | 63.36±5.49 <sup>a</sup>   | 69.72±6.01 <sup>a</sup>   |
| Sorghum Room Temperature-Dried | 18.58±0.35 <sup>b</sup> | 37.17±0.70 <sup>b</sup> | 55.75±1.05 <sup>b</sup> | 58.18±1.39 <sup>b</sup> | 67.32±2.22 <sup>b*</sup> | 73.05±2.79 <sup>b*</sup>  | 76.68±3.13 <sup>b*</sup>  | 82.95±2.87 <sup>b*</sup>  |
| Sorghum Oven                   | 14.19±1.19 <sup>c</sup> | 28.37±2.38 <sup>c</sup> | 42.56±3.58 <sup>c</sup> | 44.75±3.57 <sup>c</sup> | 53.84±4.47 <sup>a</sup>  | 58.66±4.71 <sup>a</sup>   | 61.51±5.02 <sup>a</sup>   | 67.04±5.15 <sup>a</sup>   |
| Standard Error                 | 0.71                    | 1.41                    | 2.12                    | 2.36                    | 2.89                     | 3.25                      | 3.41                      | 3.62                      |

Means with different superscripts in the same column are statistically significant, SE= standard error, ±: standard deviation (SD), (P<0.05\*, P<0.01\*\*).

**Table 4.** Methane and ammonia contents of hydroponically produced barley, wheat, corn, oat, and sorghum

| Hydroponically Produced        | Ammonia, mg/100 g       | Methane, % 24. h          | pH                      |
|--------------------------------|-------------------------|---------------------------|-------------------------|
| Barley Raw                     | 31.0±1.0 <sup>b</sup>   | 22.43±1.01 <sup>a</sup>   | 6.76±0.11 <sup>a</sup>  |
| Barley Room Temperature-Dried  | 51.0±1.0 <sup>a**</sup> | 23.10±0.26 <sup>a</sup>   | 6.80±0.05 <sup>a</sup>  |
| Barley Oven                    | 15.5±0.5 <sup>c**</sup> | 23.33±0.21 <sup>a</sup>   | 6.77±0.04 <sup>a</sup>  |
| Wheat Raw                      | 28.5±0.5 <sup>b</sup>   | 23.67±0.51 <sup>a</sup>   | 6.68±0.05 <sup>ab</sup> |
| Wheat Room Temperature-Dried   | 48.5±0.5 <sup>a**</sup> | 23.40±0.50 <sup>a</sup>   | 6.76±0.05 <sup>a*</sup> |
| Wheat Oven                     | 14.5±0.5 <sup>c**</sup> | 23.73±0.21 <sup>a</sup>   | 6.70±0.06 <sup>b</sup>  |
| Corn Raw                       | 20.5±0.5 <sup>b</sup>   | 21.33±0.40 <sup>b</sup>   | 6.44±0.02 <sup>b</sup>  |
| Corn Room Temperature-Dried    | 40.5±0.5 <sup>a**</sup> | 21.73±0.40 <sup>b</sup>   | 6.45±0.03 <sup>b</sup>  |
| Corn Oven                      | 10.5±0.5 <sup>c**</sup> | 22.17±0.25 <sup>a*</sup>  | 6.46±0.06 <sup>b</sup>  |
| Oat Raw                        | 34.5±0.5 <sup>b</sup>   | 21.70±0.87 <sup>b</sup>   | 6.91±0.01 <sup>a*</sup> |
| Oat Room Temperature-Dried     | 61.0±1.0 <sup>a**</sup> | 22.87±0.95 <sup>a*</sup>  | 6.87±0.08 <sup>ab</sup> |
| Oat Oven                       | 18.0±0.5 <sup>c**</sup> | 20.27±1.53 <sup>c*</sup>  | 6.93±0.01 <sup>a*</sup> |
| Sorghum Raw                    | 15.5±0.5 <sup>b</sup>   | 19.00±1.04 <sup>c</sup>   | 6.54±0.00 <sup>b</sup>  |
| Sorghum Room Temperature-Dried | 36.0±1.0 <sup>a**</sup> | 21.67±2.47 <sup>a**</sup> | 6.61±0.06 <sup>a*</sup> |
| Sorghum Oven                   | 8.5±0.5 <sup>c**</sup>  | 22.23±0.60 <sup>a**</sup> | 6.64±0.02 <sup>a*</sup> |
| Standard Error                 | 2.4                     | 0.23                      | 0.02                    |

Means with different superscripts in the same column are statistically significant, SE= standard error, ±: standard deviation (SD), (P<0.05\*, P<0.01\*\*).



Root elongation from day four onwards enhances mineral uptake and progressively raises the ASH and protein content of sprouts (Morgan et al., 1992; Sneath and McIntosh, 2003; Dung et al., 2010). The influence of irrigation water composition on mineral content further supports this interpretation (Fazaeli et al., 2012). The high CP values observed in oat and wheat are consistent with reports attributing CP increases in hydroponic production to carbohydrate losses during germination (Girma and Gebremariam, 2018; Boğa et al., 2023). Although total protein content does not change, its proportion on a DM basis rises as starch is consumed (Peer and Leeson, 1985; Morgan et al., 1992). Root elongation also facilitates nitrate absorption and the metabolism of nitrogenous compounds, further raising CP concentration (Morgan et al., 1992). The stable and comparatively low CP in corn (7.15–7.24%) across all treatments is consistent with this mechanism. Increases in proteolytic enzymes during germination lead to hydrolysis of prolamins and accumulation of free amino acids, with particular gains in water-soluble proteins and lysine (Sneath and McIntosh, 2003; Raeisi et al., 2018; Boğa et al., 2023). The rise in EE from raw (4.79%) to oven-dried oat (7.21%) is linked to the accumulation of structural lipids and chlorophyll during plant growth (Fazaeli et al., 2011; Shit, 2019) and this corroborates the increased lipase activity reported by Sneath and McIntosh (2003) in hydroponically produced barley. The substantial difference in CF between raw and processed samples suggests that drying consolidates cell wall components; the development of structural carbohydrates during germination is known to increase CF, NDF, and ADF while reducing nitrogen-free extracts (Shit, 2019; Boğa et al., 2023). The high NDF values in wheat and oat indicate that these grains represent high-fibre sources in ruminant nutrition, and their elevated ADF content suggests that physical processing grinding or crushing would be necessary to improve starch digestibility, given that only around 60% of unprocessed grains are digested (Sneath and McIntosh, 2003). The low ADF and lignin content of corn, by contrast, points to a structurally advantageous composition for ruminant digestibility.

#### **4.2. Organic Matter Digestibility (OMD), Metabolizable Energy (ME), Net Energy Lactation (NEL)**

Fazaeli et al. (2012) reported OMD of 87.58% in barley seed and 81.86–85.53% in hydroponically grown barley harvested on days six to eight, indicating that OMD declines with extended cultivation, likely due to progressive lignification and increasing cell wall complexity. The OMD values obtained in the present study for barley oven (65.17±1.41%) and wheat oven (66.82±2.65%) are higher than the 51.79–56.14% range reported for different barley varieties by Muruz and Çelik (2020), though lower than those of Fazaeli et al. (2012), possibly reflecting differences in grain variety, harvest timing, and drying conditions. The sharp decline in barley

OMD and energy values with room temperature-dried (OMD: 53.48±5.60%; ME: 9.29±0.15 MJ/kg DM) aligns with reports of DM reduction and increases in crude protein and water-soluble carbohydrates in hydroponically produced barley (Fazaeli et al., 2012). Starch conversion to simple sugars during germination directly affects energy content Sneath and McIntosh, (2003), and Dung et al. (2010) similarly reported a 2% gross energy loss in hydroponic barley relative to traditionally grown grain. The stability of corn OMD across drying methods (69.10–70.64%) suggests resistance to drying-related losses; comparable ME and NEL values were reported by Mahesh et al. (2024) for hydroponic corn (ME: 9.78 MJ/kg DM; NEL: 6.45 MJ/kg DM).

The low OMD of oat across all treatments (47.92–53.79%) is attributable to its high fibre content (NDF: 29.69–31.62%; ADF: 12.05–14.49%) and lignin structure, which are the principal factors limiting organic matter digestibility. Kuşan (2020) reported ME values of approximately 11.70–12.30 MJ/kg DM in hydroponic oat, considerably higher than those recorded here, a discrepancy likely explained by differences in harvest time, germination period, and drying conditions. The elevated OMD of room temperature-dried sorghum (74.75±3.20%) relative to raw and oven-dried forms suggests that ambient drying may modify cell wall structure in a manner that promotes enzymatic fermentation, though this requires further investigation. One possible mechanism underlying the improved digestibility observed in room temperature-dried sorghum may be related to alterations in cell wall architecture during the drying process. Moderate drying conditions may partially disrupt the structural organization of hemicellulose and cellulose fractions, thereby increasing the accessibility of fermentable substrates to rumen microorganisms. In contrast, exposure to higher drying temperatures may promote structural changes that reduce microbial access to these substrates and consequently limit fermentation efficiency. However, these mechanisms were not directly evaluated in the present study and therefore remain speculative.

Future studies should investigate these mechanisms using enzymatic accessibility assays, fiber degradability analyses, scanning electron microscopy, and cell wall characterization techniques. Such approaches would help clarify whether the observed differences in digestibility and energy values are associated with structural modifications occurring during the drying process and would provide a stronger mechanistic basis for the utilization of hydroponic fodders in ruminant nutrition. ME values for barley reported by Sevim et al. (2017) (11.75–12.86 MJ/kg DM) and Kuşan (2020) (approximately 11.40–13.45 MJ/kg DM) are higher than raw barley in the present study (10.72±0.57 MJ/kg DM), consistent with the general trend of declining energy content with extended hydroponic cultivation. ME values

for wheat reported by Kuşan (2020) (approximately 12.17–13.94 MJ/kg DM) are comparable to those obtained for raw wheat in the present study (12.59±0.40 MJ/kg DM). However, the lower ME value observed in oven-dried wheat (10.15±0.40 MJ/kg DM) suggests that drying may reduce the availability of fermentable nutrients, resulting in a decline in energy value. Similar reductions in energy content following hydroponic cultivation and processing have been reported in previous studies (Dung et al., 2010; Fazaeli et al., 2012). Recent studies and reviews have emphasized that digestibility and energy values of hydroponic fodders are strongly influenced by grain type, germination duration, environmental conditions, and post-harvest processing. Such variability may explain differences among studies and supports the need for species-specific evaluation of hydroponically produced feeds for ruminant nutrition (Pastorelli et al., 2024; Vastolo and Cutrignelli, 2025).

#### 4.3. In Vitro Gas Production

The high gas production of raw wheat (96.05±3.58 mL/200 mg DM at 96 h) reflects its high digestible organic matter content. Lower gas production values have been reported for hydroponically produced wheat in previous studies. Differences among studies may be attributed to variations in germination period, cultivation conditions, fibre accumulation during sprouting, and changes in plant metabolism (Sneath and McIntosh, 2003; Fazaeli et al., 2011). The decline in barley gas production from raw (81.43 mL) to room temperature-dried (73.20 mL) and oven-dried (78.52 mL) forms is consistent with findings of Fazaeli et al. (2012), who reported values of 71.33–75.45 mL/200 mg DM in hydroponic barley on days six to eight, confirming that extended cultivation reduces gas production through increased lignification. Values reported by Muruz and Çelik (2020) for different barley varieties (42.23–46.51 mL/200 mg DM) were considerably lower than those in the present study, highlighting the influence of genotype on fermentation characteristics. The stability of corn gas production across treatments (75.79–77.79 mL/200 mg DM) is consistent with its low NDF (9.8%) and ADF (3.6%) and high starch content (NRC, 2021). The elevated gas production of room temperature-dried sorghum (82.95±2.87 mL) relative to raw (69.72 mL) and oven-dried (67.04 mL) forms suggests that enzymatic activity during ambient storage may convert starch into more readily fermentable substrates, a finding consistent with the higher OMD observed in the same group. The consistently low gas production of oat (51.79–62.32 mL/200 mg DM) reflects its high fibre content (NDF: 73.6%; ADF: 39.6% per NRC, 2021) and low digestible organic matter, corroborating the observation of Sneath and McIntosh (2003) that increasing fibre content during germination reduces digestibility an effect particularly pronounced in grains already high in structural carbohydrates (Sneath and McIntosh, 2003; Boğa et al., 2023).

#### 4.4. Methane, Ammonia, and pH

The high ammonia production observed in room temperature-dried samples, particularly oat room temperature-dried (61.0±1.0 mg/100 g) and barley room temperature-dried (51.0±1.0 mg/100 g) indicates accelerated protein degradation under ambient conditions, consistent with the increased proteolytic activity reported during germination in hydroponic systems (Morgan et al., 1992; Sneath and McIntosh, 2003; Boğa et al., 2023). The marked reduction in ammonia under oven conditions (wheat oven: 14.5±0.5 mg/100 g; sorghum oven: 8.5±0.5 mg/100 g) suggests that high temperature inhibits proteolytic enzyme activity or causes protein denaturation, in line with findings of Naik et al. (2015) and Raeisi et al. (2018). The high methane production recorded in wheat samples (23.40–23.73%) is consistent with their relatively low fibre fractions and greater availability of fermentable carbohydrates. Rapid fermentation of soluble carbohydrates by rumen microorganisms may enhance methane formation, whereas the lower methane production observed in sorghum and oat samples may be associated with reduced substrate availability and higher structural carbohydrate content (Sneath and McIntosh, 2003; Boğa et al., 2023).

pH values were more strongly influenced by grain type than by drying treatment. The lowest values in corn (6.44–6.46) and the highest in oat (6.87–6.93) indicate differences in fermentation characteristics among grain sources. Similar variations in rumen pH associated with forage type and fermentation profile have been reported in previous studies, while Wang et al. (2022) reported lower values for hydroponic wheat (pH 6.04–6.17), suggesting that cultivation conditions, particularly nutrient solution composition and germination period, can substantially influence rumen fluid pH. In addition to their nutritional value, hydroponic fodders have recently attracted attention because of their potential contribution to sustainable livestock production systems. Previous studies demonstrated that hydroponically produced barley forage can be incorporated into ruminant diets without adverse effects on animal health and productivity, although responses may vary according to forage quality and dietary inclusion level. These findings support the importance of evaluating rumen fermentation characteristics when considering hydroponic feeds for practical ration formulation (Ceci et al., 2023; Ma et al., 2024). The present study contributes additional comparative information regarding the feeding value of hydroponically produced grain sources by evaluating five grain species under identical cultivation, processing, and analytical conditions. The simultaneous assessment of nutrient composition, digestibility, rumen fermentation characteristics, and methane production provides a more comprehensive comparison of their potential use in ruminant nutrition than evaluation of individual parameters alone.

## 5. Conclusion

This study demonstrated that grain type and drying method significantly influence the nutritional value, digestibility, and rumen fermentation profile of hydroponically produced cereals. Among the grains evaluated, wheat showed the highest organic matter digestibility and energy values, making it a strong candidate for energy-dense ruminant rations. Oat, despite its elevated crude protein content, showed lower energy values than the other grain sources, likely due to its high fibre fractions. Barley and corn performed at intermediate levels, though both were sensitive to drying-related losses in digestibility. In terms of rumen fermentation, wheat and barley generated considerably higher methane, whereas sorghum and oat were associated with lower emissions, a distinction attributable to differences in fermentable carbohydrate composition. Room temperature-dried consistently increased ammonia-nitrogen production across all grains, while oven drying suppressed proteolytic activity and reduced nitrogen losses. Taken together, the findings suggest that hydroponically grown cereals may serve as promising supplementary feed resources in ruminant nutrition; however, their practical value should be confirmed through *in vivo* studies. A limitation of the present study is the absence of ungerminated grain controls, which restricts direct assessment of the specific effects of hydroponic germination on nutritional composition, digestibility, and rumen fermentation characteristics. In addition, the findings are based exclusively on *in vitro* measurements and therefore do not fully reflect animal responses under practical feeding conditions. Consequently, *in vivo* studies are required to confirm the effects of hydroponically produced fodders on feed intake, nutrient utilization, animal performance, and methane emissions. Future research should address optimum harvest timing, nutrient solution composition, *in vivo* performance outcomes, and the economic viability of incorporating mixed grain combinations into practical ration formulations.

## Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

|     | M.B. | H.N.K.Ö. |
|-----|------|----------|
| C   | 50   | 50       |
| D   | 50   | 50       |
| S   | 50   | 50       |
| DCP | 50   | 50       |
| DAI | 50   | 50       |
| L   | 50   | 50       |
| W   | 50   | 50       |
| CR  | 50   | 50       |
| SR  | 50   | 50       |
| PM  | 50   | 50       |
| FA  | 50   | 50       |

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

## Conflict of Interest

The authors declared that there is no conflict of interest.

## Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

## Acknowledgements

The authors express their gratitude to the Niğde Ömer Halisdemir University Scientific Research Projects Coordination Unit for the technical support provided during the experimental and analytical phases of this study. This study was financially supported by the Niğde Ömer Halisdemir University Scientific Research Projects Coordination Unit under grant number TGT2021/21BAGEP.

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