

# Biomass Yield, Proximate Composition, *In Vitro* Gas Production and Nutritional Values of Sprouted Barely Grains on Rice, Wheat and Bean Straws

ISSN: 2770-6729



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## Abstract

Barley Grain (BG) was sprouted using agricultural by-products such as Rice Straw (RS), Wheat Straw (WS), and Bean Straw (BS) as bedding media. The sprouted barley treatments were Sprouted Barley on Rice Straw (SBRS), Sprouted Barley on Wheat Straw (SBWS) and Sprouted Barley on Bean Straw (SBBS). Irrigation was carried out with a 0.5% urea aqueous solution via an automated fogging system using a digital timer. The Dry Matter (DM) content was almost similar for the different ingredients. While BG recorded the highest contents of Organic Matter (OM), Crude Protein (CP), Ether Extractable (EE) and Nitrogen-Free Extract (NFE), whereas, RS, WS, and BS had the higher contents of Crude Fiber (CF), Fiber Fractions (NDF, ADF, ADL, cellulose and hemicellulose) and ash. Comparison of the proximate composition of different straw types showed that WS had higher contents of OM, NFE, CF, NDF, ADF, cellulose, and hemicellulose, RS had higher ash content and BS had higher contents of CP and ADL. Both fresh and dry crop biomass were significantly higher ( $P < 0.05$ ) in SBBS compared to SBWS, with slight differences with SBRS. Furthermore, SBBS had significantly ( $P < 0.05$ ) higher contents of DM and ADL, SBWS had higher contents of OM, NFE, CF, NDF, ADF and cellulose, and SBRS had higher contents of ash and hemicellulose. Meanwhile, CP and EE contents were almost similar for different sprouted barley grains. Also, Total Digestible Nutrients (TDN) and Digestible Crude Protein (DCP) contents tended to increase in SBWS, with slight differences ( $P > 0.05$ ) with both SBRS and SBBS. Accumulative gas production during the different incubation times was significantly higher ( $P < 0.05$ ) in SBRS followed by SBWS, but SBBS values were lower. Gas production also increased sharply in the first 12 hours of incubation, then increased steadily from 12 to 24 hours, and then increased slightly to 96 hours. The gas production kinetic parameters of the rapidly degradable fraction

- The potentially degradable fraction
- And the gas production rate
- Were significantly higher ( $P < 0.05$ ) in SBRS followed by SBWS, but SBBS had the lowest values.

Gas Production from Fermentation of the Soluble Fraction (GPSF) and insoluble fraction (GPNSF) were significantly higher ( $P < 0.05$ ) in SBRS followed by SBWS and lower values were in SBBS. Methane production during the different incubation times was significantly lower ( $P < 0.05$ ) in SBRS, followed by SBWS, but SBBS had higher values. Furthermore, methane production increased sharply during the first 12 hours of incubation, then steadily increased during the period from 12 to 24 hours and increased slightly as the incubation period extended to 96 hours. Short Chain Fatty Acids (SCFA) concentration and Protein Production (MCP) were significantly higher ( $P < 0.05$ ) in SBRS followed by SBWS, while it was lower in SBBS. Predicted Dry Matter Intake (DMI) expressed in kg/day or kg/kg metabolic Live Body Weight (LBW<sup>0.75</sup>), Effective Dry Matter Degradability (EDMD) and Organic Matter Digestibility (OMD) were significantly higher ( $P < 0.05$ ) in SBRS followed by SBWS, but SBBS recorded the lowest values. Gross Energy (GE) was significantly ( $P < 0.05$ ) higher in SBWS and SBBS than that of SBRS. Metabolizable Energy (ME) and Net Energy (NE) were significantly higher ( $P < 0.05$ ) in SBRS followed by SBWS, while SBBS recorded the lowest values. Whereas Digestible Energy (DE) contents tended to increase in SBWS with insignificant ( $P > 0.05$ ) with both SBRS and SBBS. From these results, it can be concluded that sprouting barley grains on rice, wheat, and bean straws is a good source of green fodder and contributes to the

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Submission: 📅 July 18, 2025

Published: 📅 August 05, 2025

Volume 4 - Issue 1

**How to cite this article:** Hamed H Gaafar\*, Reda A Mesbah, Abd EH Ghoneim and Essam M El-Kotamy. Biomass Yield, Proximate Composition, *In Vitro* Gas Production and Nutritional Values of Sprouted Barely Grains on Rice, Wheat and Bean Straws. Clin Res AnimSci. 4(1). CRAS. 000576. 2025  
DOI: [10.31031/CRAS.2025.04.000576](https://doi.org/10.31031/CRAS.2025.04.000576)

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utilization of poor-quality agricultural wastes in ruminant nutrition. Sprouting barley grains on straws increased biomass production, improved proximate composition and nutritional values and energy contents, increased feed intake, rumen fermentation, and microbial protein production, in addition to reduced methane emissions, with the best results achieved with rice straw.

**Keywords:** Sprouted barley grain; Rice; Wheat and bean straws; Composition; Biomass yield; Nutritional values; *in vitro* gas production

## Introduction

This overview highlights the potential of using agricultural by-products as a feed source for ruminants, emphasizing that large quantities of agricultural by-products are produced annually, but are not fully utilized as animal feed, leading to waste and pollution [1]. In Egypt, the area cultivated with rice is about 1.62 million feddan, producing about 4.86 million tons of rice straw, wheat is about 3.17 million feddan, producing about 6.34 million tons of wheat straw and faba beans is about 111.53 thousand feddan, producing about 167.30 thousand tons of bean straw [2]. Rice straw is of poor nutritive value for ruminants related to its low protein content, high fiber content and low palatability. Few attempts were tried to improve nutritive value of rice straw [3]. Sprouting barely increased the Crude Protein (CP) content while decreasing Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) in rice straw [4]. Sprouting activities in the seeds have many changes as in seed protein converted to essential amino acids, carbohydrates are converted to sugars and fats are converted to essential fatty acids. These activities increase because of increasing enzymes levels [5]. Sprouting is a simple, well-known technique that is used to germinate seeds of various forage crop species such as oats, barley, corn and wheat to produce valuable green fodder [6]. The sprouting process is characterized by its high yield, sustainable production throughout the year, efficient water use [7] and short growth time compared to field-grown forage production [8].

Moreover, sprouting uses 99% less land than that required for conventional production methods [4], so it is valuable for countries that have little available land for cultivation or unsuitable land. Furthermore, the seed enzymes are activated by sprouting, as they change the starch, protein and lipids within the grain to sugars, amino acids, and essential fatty acids, respectively, which are simpler forms for consumption [7]. This explains why sprouts are much easier for animals to digest than dry seeds or other conventional fodder [7]. One of the main disadvantages of sprouting is the decrease in dry matter and the increase in moisture content compared to those produced by traditional methods [9,10], which may negatively affect the consumption responses of animals [11]. In this respect, Akbag et al. [12] suggested that adding hay to sprouts would decrease the moisture content and increase the dry matter content. Sprouting is an Innovative method for production green fodder throughout the year to provide consistent high amount of food 100% organic and the systems of environment friendly technologies as well as reduce the cost of feeding ruminants [13].

Sprouted barley grain refers to barley that has been germinated by soaking in water and allowed to sprout in a controlled environment, often using hydroponics. This process enhances the nutritional value of the grain, particularly increasing enzyme

content and making it easier to digest. Sprouted barley is a valuable feed ingredient for various livestock, including poultry, rabbits, and ruminants [14]. Cereal and legume straws differ significantly in their chemical composition, particularly in protein and fiber content. Legume straws generally have higher protein content and lower fiber content compared to cereal straws [15]. The study aimed to determine the biomass production, chemical composition, nutritional values and gas production parameters in the laboratory of barley grains germinated on rice, wheat and bean straws.

## Materials and Methods

### Production of sprouted barley

Using a hydroponic watering system with straws for the process of barely germination, during one period of 12 days was done to get shoot sprouts with 15-20cm of length. The hydroponic system was kept at control room temperature maintained at 21-22 °C and 75% of the relative humidity. A growing plan was conducted using a steel hydroponic stand, size 2.10×0.50×1.9m equipped containing 6 shelves (30cm apart shelves) with capacity of 42 polyethylene trays sized 60×30×3cm (0.18m<sup>2</sup>) each. Barley grains were washed and soaked in tap water overnight (about 12 hours) before sowing and stored in a dark area to allow for initial germination. With a seed rate of 2.78kg/m<sup>2</sup> for barley grains, the seeding rates were around 500 grams of barley grains per tray [16]. Sprouted barley was produced according to the described method using about 10cm thick layer of chopped straws [17]. Sprouted Barley grain (SB) was produced using agricultural by-products such as Rice Straw (RS), Wheat Straw (WS) and bean (faba bean) straw (BS) as bedding media for growing Barley Grain (BG). Barley grain was washed and soaked in tap water overnight (about 12 hours) before sowing and stored in a dark area to allow for initial germination. The irrigation of different shelves was designed depending on fog system. The irrigation with 0.5% urea solution was delivered via 4 fog sprayers (32L/hour) for each shelf. The fog system was automated by using a digital timer (2 minutes/hour/24 hours) to control water pumping (water pump 0.5 horse powers) from the water tank. Sprouted barley treatments were Sprouted Barley on Rice Straw (SBRs), Sprouted Barley on Wheat Straw (SBWS) and Sprouted Barley on Bean Straw (SBBS).

### Proximate analysis

The fresh crops were weighed and the samples were taken and dried in the air oven at 60 °C until complete drying to estimate the dry matter content and dry crop yield. Representative samples were ground and chemically analyzed to determine the various components according to AOAC [17]. Fiber fractions (NDF, ADF and ADL) were determined according to the methods of Van Soest [18].

$$\text{Cellulose} = \text{ADF} - \text{ADL}$$

Hemicellulose=NDF-ADF

### Nutritional values

Total Digestible Nutrient (TDN) was computed according to AAFCO [19]:

$$\text{TDN \%} = 40.2625 + 0.1969 * \text{CP} + 0.4228 * \text{NFE} + 1.1903 * \text{EE} - 0.1379 * \text{CF}$$

Digestible Crude Protein (DCP) was calculated according to Villamide and Fraga [20]:

$$\text{DCP \%} = 0.876 * \text{CP} - 3.467$$

Where: CP=Crude Protein; CF=Crude Fiber; EE=Ether Extract; NFE=Nitrogen-Free Extract.

Gross Energy (GE) and Digestible Energy (DE) were calculated according to MAFF [21]:

$$\text{GE (Mcal/kg DM)} = (0.226 * \text{CP} + 0.177 * \text{CF} + 0.407 * \text{EE} + 0.192 * \text{NFE}) / 4.184$$

$$\text{DE} = (\text{GE} * (87.9 - (.88 * \text{CF}))) / 100$$

### In vitro gas production

The process outlined by Menke and Steingass [22] was followed for the *in-vitro* gas production evaluation. Briefly, 100mg of the air-dry feed ingredients were precisely weighed into a 50ml glass syringe that was calibrated and had plungers *in vitro* gas production, the buffer solution was employed [23]. The buffer comprised 2.8g of NaCl, 0.1g of CaCl<sub>2</sub>, 0.1 MgSO 4.7 H<sub>2</sub>O, 2.0g of KH<sub>2</sub>PO<sub>4</sub>, and 6.0g of Na<sub>2</sub>HPO<sub>4</sub> that were dissolved in 1L of distilled water. Next, after adjusting the pH to 6.8, CO<sub>2</sub> flushed for 15 minutes. Three rumen-cannulated lambs fed ad libitum with a mixture of commercial concentrate and rice straw were used to collect the rumen contents (50 percent liquid and 50 percent solid) according to Bueno et al. [24]. Before the animals were fed in the morning, the contents of their rumen were gathered. Both liquids and solids samples were brought to the lab in anaerobic conditions after being placed in insulated flasks preheated to 39 °C. Squeezed through four cheesecloth layers, the rumen contents were maintained in a water bath with CO<sub>2</sub> saturation at 39 °C until inoculation. In a water bath held at 39 °C with CO<sub>2</sub> saturation, the buffer and inoculant (2:1v/v) were combined [25-27]. After pipetting 15ml of buffered rumen fluid containing the feed samples into each syringe, the syringes are dropped right into the water bath, which is set at 39 °C. For every experiment, three runs were carried out. Each run consisted of two syringes filled with buffered rumen fluid, which were then incubated and used as the blank. The syringes are gently shaken every two hours, and the incubation is stopped when the 96-hour gas volume is recorded. The gas generation was measured after incubation for 3, 6, 9, 12, 24, 48, 72 and 96 hours in accordance with the procedure described by Theodorou et al. [28] linear regression analyses were determined for headspace gas pressure versus gas volume. To ascertain the methane (CH<sub>4</sub>) concentration, 10µl of the headspace gas was extracted from the bottles at various incubation durations (3, 6, 9, 12, 24, 48, 72 and 96 hours) and injected straight

into a GC, following the procedure outlined by Pellikaan et al. [29]. The reported gas and methane readings are expressed per 200mg of DM, and the total gas values are adjusted for the blank incubation. Ørskov and McDonald [30] defined fermentation kinetics as follows:

$$Y = a + b * (1 - e^{-ct})$$

Where: a is the gas production from the immediately soluble fraction; b is the gas production from the insoluble fraction; c is the gas production rate constant for fraction b. Y is the gas production (ml/g OM) at time (t).

Gas Produced after 3 hours (GP3) of incubation was employed as a new method to measure Gas Production Induced by Fermentation of the Soluble Fraction (GPSF) to evaluate feeds from those parameters. According to van Gelder et al. [31], the gas produced between the hours of GP3 and GP24 of incubation might be utilized to estimate the Gas Production due to Fermentation of the Insoluble Fraction (GPNSF) as follows:

$$\text{GPSF} = (\text{GP3hr} * 0.99) - 3$$

$$\text{GPNSF} = 1.02 * (\text{GP 24hr} - \text{GP3hr}) + 2$$

Where: GPSF is the gas production from a soluble fraction (ml/g DM); GPNSF is the gas production from a non-soluble fraction (ml/200 mg DM); and GP 3hr is the net gas production (ml/200 mg DM) during the three hours.

The energy values were calculated from the amount of gas produced at 24hr of incubation with supplementary analyses of crude protein, ash and crude fat. This approach was developed by the research group in Hohenheim (Germany) and is based upon extensive *in vitro* incubation of feedstuffs [32,33].

$$\text{ME (Mcal/kg DM)} = (2.2 + 0.136 * \text{GP} + 0.057 * \text{CP}) / 4.184$$

$$\text{NE (Mcal/kg DM)} = (2.2 + 0.136 * \text{GP} + 0.057 * \text{CP} + 0.149 * \text{EE}) * 2.2 / 14.64$$

Where: ME is the metabolizable energy (Mcal/kg DM), GP is 24hr net gas production (ml/200mg DM), CP is crude protein (% of DM) and EE is ether extract (% of DM).

Organic Matter Digestibility (OMD) was calculated as established by Getachew et al. [34]:

$$\text{OMD (\%)} = 14.88 + 0.889 * \text{GP} + 0.45 * \text{CP} + 0.0651 * \text{A}$$

Where: OMD is organic matter digestibility (%), GP is 24hr net gas production (ml/200mg DM), CP is crude protein (% of DM), A is ash (% of DM).

Effective Dry Matter Degradability (EDMD) from *in vitro* gas production data can be calculated using the equation developed by Ørskov and McDonald [30]:

$$\text{EDMD} = (a + b) + ((a * b) * c) / (c + k)$$

Where, 'a' is the gas production from the soluble fraction, 'b' is the potential gas production from the insoluble fraction, 'c' is the gas production rate constant and 'k' is the rumen outflow rate, which is at the maintenance level and is equal to 2% per hour.

The following formula was used to determine Short Chain Fatty Acids (SCFA) in accordance with Getachew et al. [33]:

$$\text{SCFA(Mm)}=(-0.00425+0.0222*\text{GP}24\text{hr})*100$$

Where: GP is the soluble fraction's net gas production over 24 hours (ml).

The expected Dry Matter Intake (DMI) of silage was computed using the formula below, in accordance with Blümmel and Ørskov [35]:

$$\text{DMI}=1.66+0.49*a+0.0297*b-4*c$$

Where: c is the gas production rate (ml/hr), a is the gas production from the soluble fraction (ml) and b is the gas production from the insoluble fraction (ml).

Czerkawski [36] calculated the generation of microbial protein (MP) as 19.3g microbial nitrogen per kilogram OMD.

$$\text{MCP(g/kg DM)}=\text{OMD}*19.3*6.25/100$$

### Statistical analysis

Data was analyzed using general linear model's procedure adapted by IBM SPSS Statistics [37] for user's guide with one-way ANOVA. Significant differences in the mean values among dietary

treatments were analyzed by Duncan's tests within SPSS program set at the level of significance  $P<0.05$  [38].

## Results and Discussion

### Proximate composition of ingredients

Proximate composition of barley grain and different straws in Table 1 showed that DM content was nearly similar for different ingredients. The proximate composition also revealed that Barley Grains (BG) recorded the highest contents of OM, CP, EE and NFE and lower contents of CF, ash, NDF, ADF, ADL, cellulose and hemicellulose compared to different types of straws (RS, WS and BS). Comparison of the proximate composition of RS, WS and BS revealed higher OM and NFE contents in WS than those in RS and BS, while higher ash content was done in RS than those in WS and BS. Whereas, higher CP content and lower EE content were observed in BS than those in RS and WS. Furthermore, higher contents of CF, NDF, ADF, cellulose and hemicellulose were detected in WS compared with RS and BS, while higher ADL content was observed in BS than those in RS and BS. Barley grains have a high starch content, about 60% (55-63% of DM), protein content (about 11-12% with values ranging from 9.5 to 13% of DM) and fiber content (4-6% CF, 5-7% ADF, 18-24%) [39] and is an important feed ingredient for major livestock species [40].

**Table 1:** Proximate composition and fiber fractions of barley grain, rice, wheat and bean straws.

Items	Barley Grain	Rice Straw	Wheat Straw	Bean Straw
<b>Proximate Composition</b>				
DM %	90.8	90.65	91.15	89.95
<b>Composition of DM %</b>				
OM	97.33	81.86	93.22	91.07
CP	11.45	3.35	3.5	7.35
CF	5.18	36.54	41.46	41.48
EE	2.14	1.43	1.42	1.36
NFE	78.56	40.54	46.84	40.88
Ash	2.67	18.14	6.78	8.93
<b>Fiber Fractions %</b>				
NDF	20.65	69.24	77.56	69.65
ADF	6.82	44.65	50.23	48.58
ADL	1.16	4.95	7.26	9.36
Cellulose	5.66	39.7	42.97	39.22
Hemicellulose	13.83	24.59	27.33	21.07

Straw is high in fiber and low in other nutrients, including protein, sugars, minerals and vitamins. Generally, straw from cereals and grasses are lower in nutritional value than straw from legumes. The leaf-to-stem ratio, which is influenced by harvesting conditions, can largely determine the nutritional value of straw, especially for legume species and should be avoided leaf shedding if the straw is used as animal feed [41]. Cereal and legume straws differ significantly in their chemical composition, particularly in protein and fiber content. Generally, legume straws have a higher protein content and lower fiber content compared to cereal straws [42]. Rice straw is a good source of energy but is low in protein

(2-5%) and its high silica content results in low digestibility and it is considered as a low-quality roughage [43]. The composition of bean straw depends on the proportions of stems and leaves: Stems have a low protein content (8% and 4% DM, respectively) while the leaves are much richer in protein (20% of DM) [44]. Bean straw contains about 5-11% protein of DM and is rich in fiber (crude fiber 38-45% DM). However, it has better nutritive value than cereal straws due to a higher protein and a lower fiber content, though the composition is variable: for instance, the DM of stem-rich bean straw was reported to contain 61% NDF vs. 51% for leaf-rich straw [40].

### Biomass yield of sprouted barley grains on straws

The yield of sprouted barley grain in Table 2 revealed that fresh and dry crop yield were higher significantly ( $P<0.05$ ) for SBBS compared with SBWS with insignificant differences with SBRS. Fresh yield ranged from 7.45 to 7.80kg and dry yield ranged from 1.76 to 1.93kg per one kg barley grains. Similar results showed that the concept of putting one kilogram of grain into a hydroponic system and producing 6 to 10 kilograms of fresh green sprouts, independent of weather and at any time of year, is of interest by Kruglyakov [44]. Commercial sprouts growers have reported that 1kg of seed yielding about 6-10kg of fresh sprouts, while trial yields from experiments found that 1kg of seed yielding about 5-8kg of

fresh sprouts, indicating an increase in fresh weight with sprouting of grain [45]. Saidi and Abo Omar [11] showed that the green fodder yield in the 8 days germination cycle was 7.5kg per 1kg barley grains of green fodder. The fresh and dry yield of sprouted barley in our study tended to increase than those obtained by Mohsen et al. [46] who found that fresh yield of sprouted barley on rice, wheat and bean straws ranged from 6.50 to 6.89kg and dry yield from 1.52 to 1.72kg per one kg grains. Also, Emam [47] reported that fresh yield of sprouted barley grains varied from 6.2 to 6.5kg per one kg grain. The amount of green fodder yield ranged from 1.95 to 2.30kg per 100g of barley grain plus 500g of media (rice straw, Acacia saligna and 50% rice straw + 50% Acacia saligna) at 15 days [48].

**Table 2:** Biomass yield, proximate composition and fiber fractions of sprouted barely grains on rice, wheat and bean straws.

Item	SBRS	SBWS	SBBS	±SE
<b>Biomass Yield (kg/kg grains)</b>				
Fresh yield	7.60 <sup>ab</sup>	7.45 <sup>b</sup>	7.80 <sup>a</sup>	0.08
Dry yield	1.85 <sup>ab</sup>	1.76 <sup>b</sup>	1.93 <sup>a</sup>	0.03
<b>Chemical Composition %</b>				
DM	24.28 <sup>ab</sup>	23.65 <sup>b</sup>	24.70 <sup>a</sup>	0.21
OM	89.13 <sup>b</sup>	94.81 <sup>a</sup>	93.74 <sup>a</sup>	1.05
CP	17.25	17.12	17.37	0.12
CF	24.64 <sup>b</sup>	27.10 <sup>a</sup>	27.11 <sup>a</sup>	0.44
EE	2.37	2.37	2.34	0.02
NFE	44.88 <sup>b</sup>	48.23 <sup>a</sup>	46.93 <sup>ab</sup>	0.58
Ash	10.87 <sup>a</sup>	5.19 <sup>c</sup>	6.26 <sup>b</sup>	0.86
<b>Fiber Fractions %</b>				
NDF	40.98 <sup>b</sup>	43.06 <sup>a</sup>	41.08 <sup>b</sup>	0.46
ADF	24.60 <sup>b</sup>	27.39 <sup>a</sup>	26.57 <sup>a</sup>	0.45
ADL	4.30 <sup>c</sup>	5.45 <sup>b</sup>	6.50 <sup>a</sup>	0.33
Cellulose	20.31 <sup>b</sup>	21.94 <sup>a</sup>	20.07 <sup>b</sup>	0.35
Hemicellulose	16.38 <sup>a</sup>	15.67 <sup>a</sup>	14.51 <sup>b</sup>	0.3

a, b, c: Means in the same row with different superscripts differ significantly at  $P<0.05$ .

### Proximate composition of sprouted barley grains on straws

Results of proximate composition of sprouted barley grains on rice, wheat and bean straws were shown in Table 2. The DM content was significantly ( $P<0.05$ ) in SBBS than that of SBWS with insignificant differences with SBRS. Whereas OM and CF contents were significantly ( $P<0.05$ ) higher in SBWS and SBBS compared to SBRS. While CP and EE content was nearly similar for sprouted barley grain on different straws. Whereas NFE content of SBWS was significantly higher than those of SBRS with insignificant differences with SBBS. Meantime, ash content was significantly ( $P<0.05$ ) high in SBRS followed by SBBS, but SBRS had the lowest content. The variations in the chemical composition of the different sprouted barley grains might be attributed to the differences in the composition of different straws as shown in Table 1. The similar CP content of different sprouted barley grains might be due to using urea water solution in irrigation. This finding may be attributed to

an increase of the activity of sprouted barley hydrolytic enzymes and lead to improvements in chemical composition of straws. Sprouting barley can significantly increase CP content of straws [49]. The chemical composition of sprouted barley differs from the original grains, which the contents of CP, CF, Ash, EE were increased whereas OM and NFE decreased ( $P<0.05$ ) in the hydroponic barley when compared with the original grain [7]. Sprouted barley on straws, as a feed, exhibits a higher content of crude protein, acid detergent fiber, lignin and ash compared to traditional diets [14]. Sprouted barely increased CP and NFE contents and decreased CF contents in treated rice and acacia straws [48]. The values of chemical composition obtained in the current study were nearly like those presented by Mohsen et al. [46].

### Fiber fractions of sprouted barley grains on straws

Results of fiber fractions in Table 2 illustrated that the contents of NDF and cellulose were higher significantly ( $P<0.05$ ) in SBWS than

those in SBRS and SBBS. While ADF content was higher significantly ( $P<0.05$ ) in SBWS and SBBS compared to SBRS. Whereas ADL content was higher significantly ( $P<0.05$ ) in SBBS than that in SBRS and SBWS. However, hemicellulose content was higher significantly ( $P<0.05$ ) in SBRS and SBWS than that in SBBS. The changes in the fiber fractions of different types of sprouted barley grain are due to differences in fiber fractions between different varieties of straws as shown in Table 1. The chemical composition of sprouted barley differs than the original grains, which the contents of NDF, ADF and ADL were increased ( $P<0.05$ ) in the hydroponic barley when compared with the original grain [7]. Sprouted barley decreased NDF, ADF and ADL contents in treated rice and acacia straws [48]. Sprouting barley can significantly alter the fiber fractions of straws, primarily reducing the content of Neutral, making them easier for animals to utilize [49].

**Accumulative gas production of sprouted barley grains on straws**

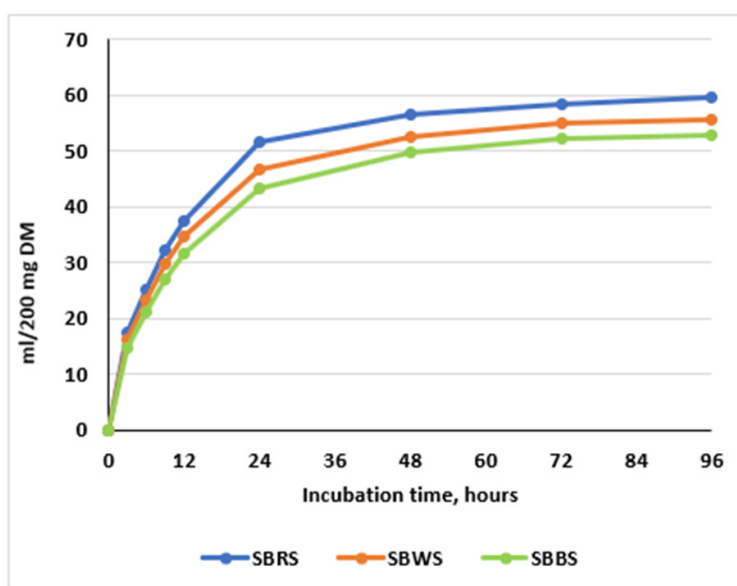
Accumulative Gas production of sprouted barley grain on different straws during the 96 hours incubation period are presented in Table 3. The values of accumulative gas production

during the different incubation times were higher significantly ( $P<0.05$ ) in SBRS followed with SBWS, but SBBS had the lower values. These results confirm the increase in gas production values with increasing NFE content and decreasing CF and fiber fractions content as shown in Table 2. Gas production increased sharply during the first 12 hours of incubation then steadily increased during the period from 12 to 24 hours and slightly increased as the incubation period extended to 96 hours (Figure 1). *In vitro* gas production from sprouted barley grain, compared to unsprouted barley, often exhibits increased levels, particularly of CO<sub>2</sub>. This is due to the breakdown of complex carbohydrates during sprouting, making them more readily available for fermentation in the *in vitro* system. Studies have shown that sprouted barley can lead to higher digestibility and improved nutrient absorption compared to unsprouted barley [50]. *In vitro* gas production experiments on sprouted barley grains, whether grown on straws or not, demonstrate that sprouting increases the digestibility of nutrients and reduces the production of methane. Sprouting enhances fermentation rates, particularly for carbohydrates and protein. Additionally, sprouted barley can be grown on various straws, including rice straw, wheat straw, and bean straw [14].

**Table 3:** Accumulative gas production (ml/200mg DM) of sprouted grains on rice, wheat and bean straws.

Incubation Time (Hours)	SBRS	SBWS	SBBS	±SE
3	17.40 <sup>a</sup>	16.09 <sup>b</sup>	14.61 <sup>c</sup>	0.44
6	25.22 <sup>a</sup>	23.31 <sup>b</sup>	21.17 <sup>c</sup>	0.64
9	32.33 <sup>a</sup>	29.89 <sup>b</sup>	27.14 <sup>c</sup>	0.83
12	37.60 <sup>a</sup>	34.75 <sup>b</sup>	31.56 <sup>c</sup>	0.97
24	51.50 <sup>a</sup>	46.61 <sup>b</sup>	43.23 <sup>c</sup>	1.25
48	56.69 <sup>a</sup>	52.65 <sup>b</sup>	49.64 <sup>c</sup>	1.08
72	58.28 <sup>a</sup>	54.87 <sup>b</sup>	52.38 <sup>c</sup>	0.92
96	59.62 <sup>a</sup>	55.50 <sup>b</sup>	52.87 <sup>c</sup>	1.04

a, b, c: Means in the same row with different superscripts differ significantly at  $P<0.05$ .



**Figure 1:** Accumulative gas production of sprouted barley grains on rice, wheat and bean straws.

By incubating substrate in buffered rumen fluid, *in vitro* Gas Production (GP) assessment is primarily used to assess the nutritional value of bovine feeds [51-53]. When feedstock is incubated *in vitro* with buffered rumen fluid, the carbohydrates are fermented to produce Short-Chain Fatty Acids (SCFA), microbial cells and mainly CO and CH gases. The fermentation of carbohydrates to acetate, propionate, and butyrate produces gas. Compared to the fermentation of carbohydrates, the creation of gas from protein fermentation is comparatively minor, and the contribution of fat to gas production is insignificant [54,55]. According to Haddi et al. [55], the rate and degree of GP were significantly correlated negatively with both ADF and NDF. Carbohydrates significantly impact the amount of gas generated during fermentation. Diets rich in soluble carbohydrates tend to produce less gas, while those with higher Water-Soluble Carbohydrate (WSC) content, such as high-WSC cultivars, can lead to greater gas production [56].

### Kinetic parameters of sprouted barley grains on straws

Kinetics parameters of gas production of sprouted grains on different straws are shown in Table 4. Kinetics parameters as gas production from the rapidly degradable fraction (a) the potentially degradable fraction (b) and the rate of gas production (c) were significantly ( $P<0.05$ ) higher in SBRS followed by SBWS, but SBBS had the lower values. The kinetics parameters (a, b, and c) for gas production in sprouted barley grain, as estimated in studies, generally show an increase in the rapid degradable fraction (a), the potentially degradable fraction (b), and the rate of gas production (c) as the sprouting process progresses. Specific values vary depending on the study and the stage of sprouting, but a general trend is an increase in the degradability of the grain's components [57]. The kinetic and nutritional parameters of gas production from sprouted barley grains when used with rice, wheat and bean straws are influenced by the straw type and the sprouting process itself [58].

**Table 4:** Kinetics parameters and fractions of gas production of sprouted barely grains on rice, wheat and bean straws.

Item	SBRS	SBWS	SBBS	±SE
<b>Kinetics Parameters</b>				
a (ml/200mgDM)	6.94 <sup>a</sup>	6.13 <sup>b</sup>	5.29 <sup>c</sup>	0.26
b (ml/200mgDM)	47.14 <sup>a</sup>	44.31 <sup>b</sup>	40.95 <sup>c</sup>	0.99
c (ml/hour)	0.073 <sup>a</sup>	0.065 <sup>b</sup>	0.055 <sup>c</sup>	0.003
<b>Gas Fractions</b>				
GPSF (ml/200mgDM)	14.31 <sup>a</sup>	12.52 <sup>b</sup>	11.37 <sup>c</sup>	0.45
GPNSF (ml/200mgDM)	36.78 <sup>a</sup>	33.14 <sup>b</sup>	31.20 <sup>c</sup>	0.83

a, b, c: Means in the same row with different superscripts differ significantly at  $P<0.05$ .

The kinetic parameters (a, b, and c) of gas production from sprouted barley grain and straw refer to the rate and extent of gas production during fermentation. 'a' represents the immediately degradable fraction, 'b' the potentially degradable fraction, and 'c' the degradation rate. These parameters, when combined, help understand how quickly and to what extent a substrate can be broken down by microbes during digestion, such as in the rumen of ruminants [57]. Sprouted barley, due to the enzymatic breakdown of its cell walls during sprouting, has a higher 'a' fraction (immediately degradable) compared to unsprouted barley or straw. This leads to faster and more extensive gas production. Straw, with its higher fiber content, has a lower 'a' fraction but a potentially higher 'b' fraction that is broken more slowly [14]. The parameters (a, b, and c) provide valuable insights into the fermentation characteristics of sprouted barley and straw, helping to understand their nutritional value and digestibility in animal feeds, particularly in ruminant diets [50]. The kinetics of gas production from sprouted barley grains on straw involve a few key parameters: gas production rate (c), total gas production (A), and time to produce a certain percentage of total gas production. Barley grain typically shows a higher gas production rate and total gas production compared to straw, with a shorter time to produce a given percentage of total gas [58]. The soluble fraction, insoluble fraction, and degradation rate are the three characteristics that now characterize ruminant forages [59].

### Gas fractions of sprouted barley grains on straws

Gas Production from Fermentation of the Soluble Fraction (GPSF) and Insoluble Fraction (GPNSF) were higher significantly ( $P<0.05$ ) in SBRS followed by SBWS, the lower values were in SBBS (Table 4). In the context of animal feed, sprouted barley grain and straw are often evaluated using gas production techniques to assess their digestibility and nutritional value. Gas Production Fractions (GPSF) and Potential Gas Production (GPNSF) are key metrics in these assessments. Sprouting barley increases the amount of fiber, which may lead to a higher potential for gas production than unsprouted barley [60]. Gas Production Fractions (GPSF and GPNSF) refer to the amount of gas produced by rumen microorganisms during the fermentation of feedstuffs. Sprouted barley grain on straw, when fed to ruminants, influences these fractions. The sprouted barley grain itself contributes to the overall gas production, while the straw can also be a source of gas production depending on its digestibility and the presence of microorganisms [61]. In essence, sprouted barley grain is likely to contribute more to gas production than straw due to its higher digestibility and starch content. However, the specific amount of gas produced from both feedstuffs will depend on a variety of factors, including the type of straw, its digestibility and the overall diet composition [62]. Gas production from soluble and insoluble substances differs significantly due to their different interactions with solvents and digestion processes.

Soluble substances, like some sugars and gases, are readily dissolved and can be more easily broken down, potentially leading to more rapid gas production. Insoluble substances, such as certain fibers, require bacterial fermentation in the large intestine, which can also produce gas, but often at a slower rate [63]. The type of substance (soluble or insoluble) and its digestibility, along with the activity of gut bacteria, are key factors influencing the rate and extent of gas production [64].

### Methane production (CH<sub>4</sub>)

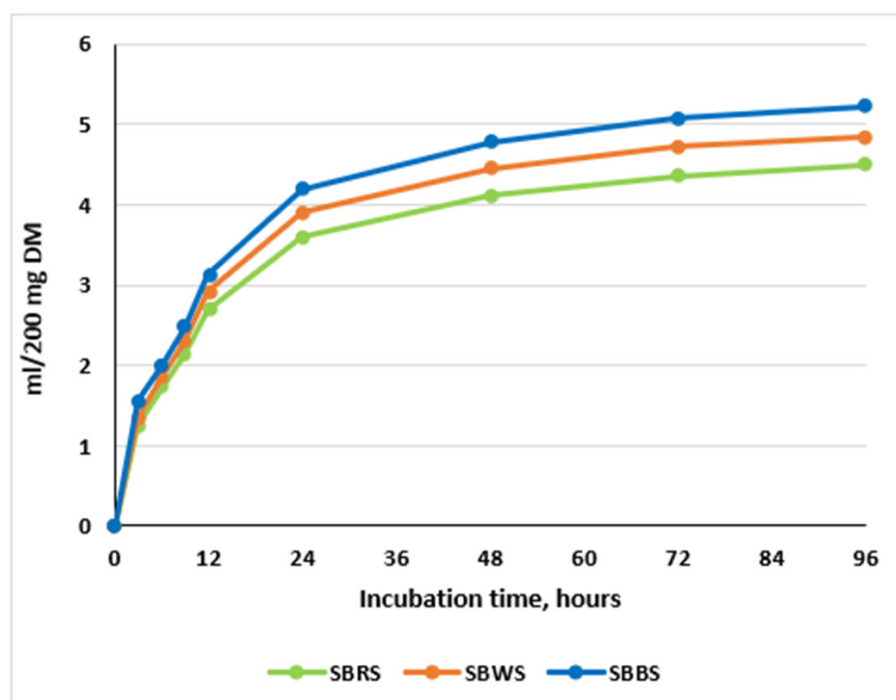
Methane production of sprouted barley grain on different straws during the 96 hours incubation period are presented in Table 5. Values of methane production during the different incubation times were lower significantly ( $P < 0.05$ ) in SBRS followed with SBWS, but SBBS had the higher values. These results confirm the increase of methane production values with increasing CF and fiber fractions contents as shown in Table 2. Methane production increased sharply during the first 12 hours of incubation then steadily increased

during the period from 12 to 24 hours and slightly increased as the incubation period extended to 96 hours (Figure 2). *In vitro* methane production from sprouted barley grain on straw is influenced by several factors, including the specific conditions of the *in vitro* incubation, the composition of the substrate (sprouted barley and straw) and the microbial community present. Studies have shown that sprouted barley can alter methane production compared to using barley grain alone and the type of straw used (e.g., rice straw) can also play a role [65]. The type of straw used as a substrate can impact methane production. For example, rice straw has been used in studies involving sprouted barley and its properties (e.g., particle size, amount relative to grain) can influence fermentation and methane release [66]. *In vitro* methane production from sprouted barley on straw is a complex process influenced by various factors. Understanding these factors is crucial for optimizing the use of sprouted barley and straw as feed while minimizing methane emissions [67].

**Table 5:** Methane production (ml/200mgDM) of sprouted grains on rice, wheat and bean straws.

Incubation Time (Hours)	SBRS	SBWS	SBBS	±SE
3	1.25 <sup>c</sup>	1.35 <sup>b</sup>	1.56 <sup>a</sup>	0.03
6	1.73 <sup>c</sup>	1.86 <sup>b</sup>	2.00 <sup>a</sup>	0.04
9	2.14 <sup>c</sup>	2.31 <sup>b</sup>	2.48 <sup>a</sup>	0.06
12	2.69 <sup>c</sup>	2.91 <sup>b</sup>	3.13 <sup>a</sup>	0.07
24	3.60 <sup>c</sup>	3.90 <sup>b</sup>	4.19 <sup>a</sup>	0.09
48	4.11 <sup>c</sup>	4.45 <sup>b</sup>	4.78 <sup>a</sup>	0.11
72	4.36 <sup>c</sup>	4.72 <sup>b</sup>	5.07 <sup>a</sup>	0.11
96	4.49 <sup>c</sup>	4.84 <sup>b</sup>	5.22 <sup>a</sup>	0.12

a, b, c: Means in the same row with different superscripts differ significantly at  $P < 0.05$ .

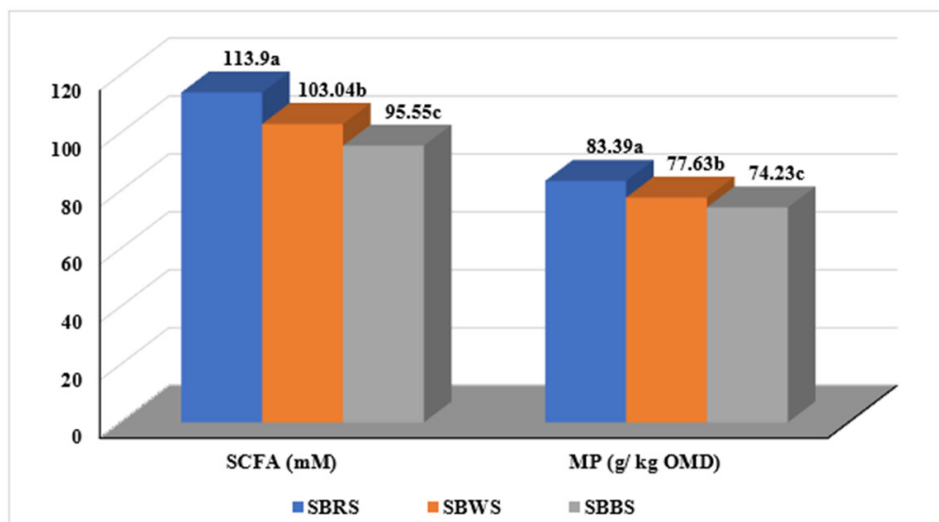


**Figure 2:** *In vitro* methane production of grains sprouted on rice, wheat and bean straws.

## Fermentation Products of sprouted barley grains on straws

**Short Chain Fatty Acids (SCFA):** Fermentation product in Figure 3, revealed that the concentration of Short Chain Fatty Acids (SCFA) was higher significantly ( $P < 0.05$ ) in SBRS followed by SBWS, whereas it was lower in SBBS. Short Chain Fatty Acids (SCFA) are the main products of fermentation, including acetate, propionate and butyrate, which are important energy sources for ruminants. Gas production is associated with volatile fatty acid production following fermentation of substrate [68]. *In vitro* Short-Chain Fatty Acid (SCFA) production involves simulating the fermentation process of gut microbiota in a lab setting to study SCFA production and its impact on health. This method allows

researchers to explore how different substrates, like dietary fiber, influence SCFA production [69]. The production of SCFA, which was predicated on the fermentation of carbohydrates, was closely correlated with the gas production of various feed classes cultured *in vitro* in buffered rumen fluid [70,71]. *In vitro* Short-Chain Fatty Acid (SCFA) concentrations vary depending on the specific SCFA, the location within the gut and the experimental conditions. Typical concentrations of SCFA, with acetate being the most abundant, followed by propionate and butyrate. *In Vitro*, studies often investigate SCFA production from different substrates and their effects on various biological processes [72]. McDonald et al. [71] have revealed that the concentration of SCFA measured in this investigation falls within the usual range of 70 to 150mM.



**Figure 3:** Short Chain Fatty Acids (SCFA) and Microbial Protein Production (MCP) of sprouted barely grains on rice, wheat and bean straws.

**Microbial Protein production:** Gas production can indicate the extent of Microbial Protein Production in the rumen (MCP) of sprouted barley grain on different straws is shown in Figure 3. The SBRS revealed significantly ( $P < 0.05$ ) higher MCP followed by SBWS, but the lower value detected in SBBS. These results might be due to more utilization of rice straw by rumen microbial. According to Lu et al. [72], rumen MCP output increases significantly when fed an energy-rich diet while remaining unaffected by a protein-rich diet. The main variables influencing the energy available for rumen microbial development (namely, MCP synthesis) are the sources and amounts of fed carbohydrates, while provided protein has an impact on the amount of microbial Dry Matter (DM) produced per unit of fermented carbohydrates [73]. Only when more Non-Fiber Carbohydrates (NFC), known as the primary energy source for ruminal microbes, are supplied to the animals will more protein N be absorbed into the rumen MCP [74]. Gas production in the rumen can be used as an indicator of Microbial Protein (MCP) production when feeding sprouted barley grain on different straws. The amount and rate of gas production are related to the extent of microbial fermentation and protein synthesis within the rumen [75]. Rumen microbes utilize feedstuffs to produce microbial protein, which is

a crucial source of amino acids for the ruminant animal. Also, gas production is a useful indicator of rumen MCP synthesis [76].

**Predicting DMI:** The prediction of Dry Matter Intake (DMI) of sprouted barley grain on different straws is shown in Table 6. The predicted DMI expressed as kg per day or kg per kg metabolic Live Body Weight (LBW<sup>0.75</sup>) were higher significantly ( $P < 0.05$ ) in SBRS followed by SBWS, but SBBS recorded the lower values. *In vitro* gas production techniques can be used to predict Dry Matter Intake (DMI) of sprouted barley grain and straw-based diets. The rate and extent of gas production, along with parameters like potential cumulative gas production (B), rate of gas production (c), and the initial lag time (lag), can be used to predict the DMI of different feeds [77]. The rate and extent of gas production (measured at various time points during incubation) can be used to predict how much of the feed an animal will consume (DMI) [78]. *In vitro* techniques can be used to assess how sprouted barley affects the digestibility and gas production of straw-based diets, allowing researchers to predict how these changes will influence DMI [79]. The concentration of cell wall components limits the amount of forage consumed [80,81]. High correlations between DMI of forages and *in vitro* GP investigations have been reported by several authors [82].

**Digestibility:** As shown in Table 6, Effective Dry Matter Degradability (EDMD) and Organic Matter Digestibility (OMD) were significantly ( $P < 0.05$ ) higher in SBRS followed by SBWS, whereas SBBS recorded the lower values. *In vitro* gas production can estimate the digestibility of Organic Matter (OMD) and Dry Matter (IVDMD). *In vitro* techniques can be used to assess how sprouted barley affects the digestibility of straw-based diets [83]. The effective degradability of Dry Matter (DM) of sprouted barley on straws is influenced by several factors, including the sprouting process itself and the potential for interactions with the straw in a mixed diet. While sprouting can improve the digestibility of barley grain, the overall effect on DM degradability when fed with straw depends on the specific conditions and proportions of the feed [84]. It is commonly recognized that the most suitable and widely used laboratory methodology for estimating the digestibility of feedstuffs for ruminants is the *in vitro* technique. According to Taghizadeh et al. [85], there was a significant positive link between gas production and *in vitro* dry matter disappearances. The sprouting process increases the digestibility of nutrients, making them more readily available for animals. Sprouted barley also shows higher levels of

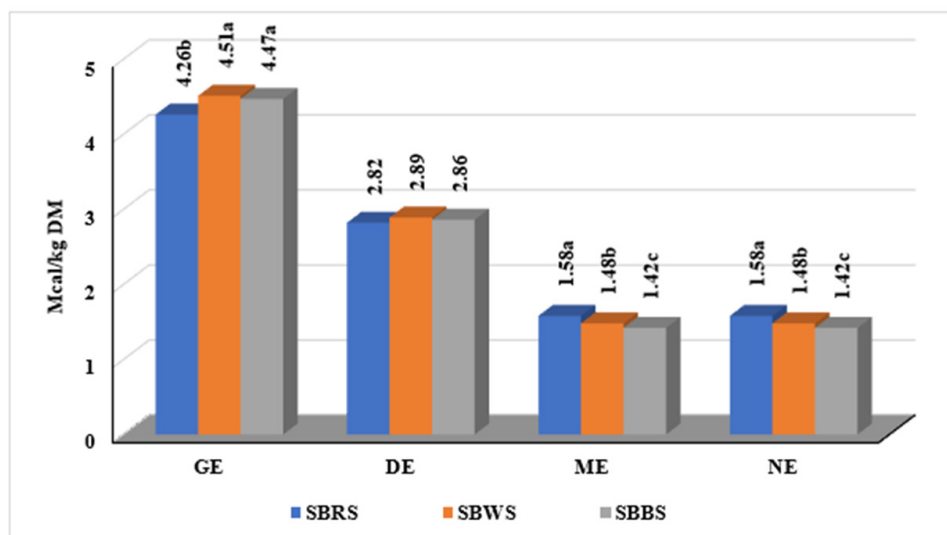
digestible nutrients compared to traditional diets [14].

**Nutritional values:** Nutritional values illustrated in Table 6 indicated that Total Digestible Nutrient (TDN), Digestible Crude Protein (DCP), Gross Energy (GE) and Digestible Energy (DE) contents tended to increase in SBWS with insignificant ( $P > 0.05$ ) with both SBRS and SBBS. These results revealed that sprouting process improved the feeding values of sprouted barley grains as calculated from chemical composition. Sprouting barley grains on different straws increased its contents of TDN by from 40-45% to 62-63%, DCP from 1.5-2.0% to 11.50 to 11.75% and seems a good quality forage for ruminants. The improvement of nutrient quality of sprouted barley due to the ability of sprouted germinated plants to transport nutrients to the roots to meet the requirement for strong healthy vegetative growth, besides increasing protein, vitamins and minerals content during process [86,87]. Sprouting barley can increase the TDN content of straw-based diets for ruminants. This happens because sprouting breaks down structural and complex carbohydrates into simpler sugars, making them easier to digest. Sprouting barley significantly alters its nutritional profile, especially when combined with rice, wheat and bean straws [88].

**Table 6:** Fermentation product and nutritional parameters of sprouted barely grains on rice, wheat and bean straws.

Item	SBRS	SBWS	SBBS	±SE
DMI (kg/day)	6.17 <sup>a</sup>	5.72 <sup>b</sup>	5.25 <sup>c</sup>	0.15
DMI (g/kg LBW0.75)	58.73 <sup>a</sup>	54.47 <sup>b</sup>	49.97 <sup>c</sup>	1.39
EDMD %	65.63 <sup>a</sup>	59.01 <sup>b</sup>	52.05 <sup>c</sup>	2.16
OMD %	69.13 <sup>a</sup>	64.36 <sup>b</sup>	61.54 <sup>c</sup>	1.17
TDN %	62.06	63.1	62.56	0.21
DCP %	11.64	11.53	11.75	0.11

a, b, c: Means in the same row with different superscripts differ significantly at  $P < 0.05$ .



**Figure 4:** Energy contents of sprouted barely grains on rice, wheat and bean straws.

**Energy values:** The effect of different straws on energy contents of sprouted barley grain are presented in Figure 4. Gross Energy (GE) was significantly ( $P < 0.05$ ) higher in SBWS and SBBS

than that of SBRS. Whereas Digestible Energy (DE) contents tended to increase in SBWS with insignificant ( $P > 0.05$ ) with both SBRS and SBBS. The contents of Metabolizable (ME) and Net Energy

(NE) were significantly ( $P < 0.05$ ) higher in SBRS followed by SBWS, but SBBS had the lower values. The ME and NE of sprouted barley grain on straw can be estimated using the *in vitro* gas production technique. This method involves measuring gas production from the feed sample during incubation, which is then used in equations to calculate ME and NEL values [87]. The metabolizable energy value of traditional feeds assessed a positive association with the protein and carbohydrates and a negative association with fiber contents and the metabolizable energy computed from a 24-hour *in vitro* gas generation [28]. The energy value of various feed classes, especially straws, has also been extensively assessed using the *in vitro* gas production method [89]. Sprouting barley grains on different straws increased GE from 3.4-3.8Mcal/kg to 4.26-4.51Mcal/kg and seems a good quality forage for ruminants [86].

## Conclusion

From these results it could be concluded that sprouting barley grain on rice, wheat and bean straws are a good source of green fodder and utilization of poor-quality agriculture by-products in ruminant feeding. Sprouting barley grain on straws increased biomass yield and improved chemical composition and nutritional values and energy contents, feed intake, rumen fermentation and microbial protein production as well as reduced methane emission with best results with rice straw.

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