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Rumen Degradability and *In Vitro* Fermentation Characteristics of Various Cereal Grains

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ABSTRACT

A study was conducted to evaluate the in vitro gas production (IVGP), degradability and fermentation characteristics of various cereal grains viz., maize, broken rice, jowar, ragi, wheat, barley and bajra by in vitro gas production technique.The in vitro gas production technique conducted indicated significant (P< 0.01) variation for cereal grains with regard to in vitro gas produced (IVGP) volume, in vitro dry matter degradability (IVDMD %), in vitro organic matter degradability (IVOMD %) and in vitro neutral detergent fibre degradability (IVNDFD %). The IVGP and IVOMD were highest for wheat and barley followed by bajra and lowest for maize, broken rice, jowar and ragi. The order of the IVDMD % from highest to lowest was broken rice> wheat>jowar and maize>bajra>barley>ragi. The IVNDFD (%) was highest for wheat and barley followed by bajra then by ragi and lowest in jowar, broken rice and maize. The partitioning factor was higher (P< 0.01) in broken rice, jowar and maize, followed by ragi, then bajra and was lowest in wheat and barley. The microbial biomass production (MBP) (mg/500mg) was lowest (P< 0.01) in maize, broken rice, jowar and ragi, followed by bajra and highest in wheat and barley. The efficiency of microbial biomass production (EMBP) (g/kg DOM) was higher (P< 0.01) and comparable for broken rice, maize and jowar followed by ragi and then bajra and lowest was from barley and wheat. The ME values from in vitro study varied significantly (P< 0.01) and in descending order was wheat >barley >bajra>broken rice >jowar and maize >ragi. The pH was highest (P> 0.01) in maize and jowar and comparable with broken rice and bajra, followed by ragi and lowest was for wheat and barley. The NH3-N concentration from various cereal grains did not vary, but total volatile fatty acid production was significant. The highest (P< 0.01) TVFA production was from wheat, followed by barley then bajra, jowar and lowest in maize, broken rice and ragi.

Key words: Cereal grains, In vitro gas production, Ruminal degradability, Ruminal fermentation

INTRODUCTION

Ruminants obtain most of their energy from cereal grains in order to meet their energy demands and fermentability of these grains largely determines the feeding value for ruminants. The fermentability of cereal grains in rumen has an important effect on the rumen environment due to changes related to ruminal pH, fibre degradability and volatile fatty acid (VFA) production (Sauvant, 1997) and ultimately affecting the intake and milk production (Herrera-Saldana *et al.,* 1990). Cereal grains like maize, rice, wheat, barley, bajra, ragi and jowar are the most important energy sources and among these, maize is the commonly used cereal grain in concentrate mixtures of livestock and poultry due to its high energy content. In our country, there is a huge gap between requirement and availability of maize for livestock and poultry feeding and majority of maize is utilized by the poultry sector (Maize Vision, 2022). Hence there is need to find alternativesfor commonly used maize grain for ruminants. Grains differ in their nutrient composition and their ruminal degradability (Humer and Zebeli, 2017). Optimal rumen environment is essential for improving fermentation endproducts for ruminants use (Wanapat, 2000). Limited information is available on characteristics on nutrient degradation in rumen of commonly available cereal grains in our country. Thus the objective of this study was to determine the degradability of dry matter, organic matter and fibre and fermentation characteristics of 7 available cereal grains using in-vitro gas production technique.

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MATERIALS AND METHODS

Maize, broken rice, ragi, barley, bajra, wheat and jowar grains were procured from local market in Hyderabad. The cereal grains were ground to uniform size by passing through 1mm sieve with Wiley mill, then they were mixed thoroughly to reduce the sampling errors. The cereal grains were analyzed for proximate constituents (AOAC, 2019) and neutral detergent fiber (Van Soest *et. al.,* 1991).

Rumen contents was collected from adult steer immediately after the slaughter and kept in warm water (39^{o}C) until it was carried to laboratory. Then the rumen contents were strained with four layers of muslin cloth and that strained rumen liquor was kept in water bath (39^{o}C)and flushed with carbon dioxide to support the liveability of anaerobic organisms.

The incubations were carried out in 100 ml glass syringes (HaberleLabortechnik, Lonsee-Ettenchieß, Germany) kept in water bath maintained at 39±0.5^{o}C as described by Menkeet al. (1979) and Menke and Steingass (1988). The amount of cereal grains and the volume of incubation medium were 500 mg and 40 ml, respectively.The medium mixture was prepared using double strength rumen buffer (Blummel *et al.,* 1997) and the ratio of medium mixture to rumen liquor was kept at 2:1. The cereal grains were incubated in triplicate and blank was set comprised of rumen fluid-medium mixture alone and was run simultaneously. The gas production was recorded at 0 h, ½ h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h and 24 h. After removing the syringes at 24 h, the pH was recorded immediately and the contents were transferred to 600 ml capacity spoutless beaker, shaken and allowed to settle. After 15 minutes, 10 ml supernatant was carefully pipetted for ammonia-N (NH3-N) estimation and 5 ml supernatant was stored with saturated mercuric chloride for total volatile fatty acids (TVFA) estimation. Then the syringes were rinsed twice with 25 ml of neutral detergent solution, double strength (Van Soestet al., 1991) and contents were transferred through narrow outlet into the respective beakers and NDF was determined (Blummel *et al.,* 1997).

The in vitro dry matter degradability (IVDMD) (%), organic matter degradability (IVOMD) (%) and neutral detergent fibre degradability (IVNDFD) (%) were determined by

IVDMD% = DM% of the substrate – DM % of the residue

IVOMD % = OM% of substrate incubated on DM basis – OM % of the residue

IVNDFD % = NDF% of substrate incubated on DM basis – NDF % of the residue

The partitioning factor (PF), Microbial biomass protein (MBP) and metabolizable energy (ME) were estimated as follows:

$$\text{Partitioning factor} = \frac{\text{In vitro true DM Digested (mg)}}{\text{Total gas produced (ml)}}$$

MBP (mg) = IVOMD (mg) – (Net gas volume × 2.20)

Where 2.20 is the stoichiometric factor (Blummel and Lebzien, 2001).

In vitro ME (MJ/kg DM) = 2.20 + 0.1356 GP + 0.057 × CPDM/kg (Menke and Steingass, 1988)

Where, GP = Net gas production at 24 h fermentation (ml/0.5 g DM);

CPDM/kg = Crude protein on DM basis × 10

The data was subjected to statistical analysis using software (SPSS, Version 17). One way analysis of variance through generalized linear model was used to analyse all the results. The treatment means were ranked using Duncan's multiple range test with a significance at $P<0.05$ (Duncan, 1955). All the statistical procedures were done as per Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The chemical composition (% DMB) of seven cereal grains is presented in table 1. The chemical composition of cereal grains varied significantly ($P<0.01$) except for the DM. The crude protein (% DMB) was highest ($P<0.01$) in wheat, followed by bajra, then by broken rice, barley and jowar and next by maize, and lowest in ragi. The CP content of maize, broken rice, barley and bajra were lower than the values given by NDDB (8-12,8-10, 11-16 and 12-15 %, respectively), while jowar and wheat are similar to the NDDB values (8-12 and 8-14%, respectively). The cereal grains accordingto ether extract (% DMB) in descending order ($P<0.01$) was bajra> maize> wheat and jowar followed by ragi>

barely andbroken rice. The EE content of maize, jowar, wheat and bajra were higher and barley is lower than the values given by NDDB,while rice is similar. The cereal grains according to crude fibre (% DMB) in descending order (P<0.01) was ragi>jowar = maize = bajra = wheat=barley and broken rice. The total ash(%DMB)was highest (P<0.01) in ragi followed by jowar> then bajra later in wheatand maize followed by barley and lowest in broken rice.The NFE content (% DMB) was highest (P<0.01) in barley and broken rice followed by jowar, maize, ragi later in wheatand lowest was observed in bajra. The NDF (% DMB) was highest(P<0.01)in ragi, followed by barely and decreased linearly in bajra, wheat and maize, then in jowar and lowest in broken rice.

Table 1. Chemical composition (% DMB) of cereal grains

Cereal grain	Dry matter	Crude protein	Ether extract	Crude fibre	Total ash	Nitrogen free extract	Neutral detergent fibre
Maize	89.58	7.44^{c}	5.45^{b}	1.26^{bc}	1.30^{d}	84.54^{b}	12.76^{d}
Broken Rice	89.32	8.75b^{c}	1.53^{e}	0.20^{d}	0.69^{f}	88.83^{a}	6.85^{f}
Jowar	90.67	7.57^{bc}	3.38^{cd}	1.81^{b}	2.02^{b}	85.22^{b}	11.31^{e}
Wheat	90.58	11.03^{a}	3.89^{c}	0.79^{cd}	1.50^{d}	82.78^{bc}	13.68^{d}
Ragi	90.57	5.71^{d}	2.94^{d}	3.78^{a}	3.99^{a}	83.59^{b}	24.61^{a}
Barley	91.94	7.90b^{c}	1.69^{e}	0.36^{d}	1.00^{e}	89.05^{a}	22.43^{b}
Bajra	90.17	9.07^{b}	6.98^{a}	1.07^{c}	1.78^{c}	81.10^{c}	18.02^{c}
SEM	0.635	0.372	0.417	0.258	0.225	0.664	1.318
P Value	0.09	0.001	0.001	0.001	0.001	0.001	0.001

Each value is an average of triplicate analysis.
[abcdef]Means with different superscripts in a column differ significantly: P<0.01
SEM: Standard Error Mean; P: Probability value

The IVGP volume (ml/500mg) significantly (P<0.01) differed among the cereal grains (Table 2).The IVGP volume was highest for wheat and barley, followed by bajra and lowest from maize, broken rice, jowar and ragi. The IVGP volume among latter 4 cereals was comparable.The gas volume produced is indicating that wheat and barley fermented faster than the other cereal grains investigated (bajra, maize, broken rice, jowar and ragi).The faster fermentation in wheat and barley grains could be due to presence of higher proportion of floury endosperm(Rooney and Pflugfelder, 1986).The present study reports are in accordance with Opatpatanatik *et al.* (1994), who reported higher gas volume with wheat than with maize grain. Amanzougarene *et al.* (2020) reported that barley produced more gas volume than the sorghum grain. But contrary to the present study results, Lanzas *et al.* (2006) reported more gas production for sorghum > corn > wheat and barley. Yoo *et al.* (2020) reported that higher gas production was with rice TMR than with corn TMR which also could be the associative effect of other ingredients present in TMR.

The in vitro degradability of DM, OM, and NDF differed significantly (P<0.01) for various cereals analysed (Table 2).

Table 2. In vitro gas production and in vitronutrient degradability of various cereal grains assessed by in vitro gas production technique

Cereal grain	IVGP (ml/500 mg feed)	IVDMD%	IVOMD%	IVNDFD%	PF
Maize	28.24±0.17[c]	93.38±0.22[c]	44.18±0.15[c]	44.38±2.11[d]	15.20±0.15[a]
Broken Rice	28.00±1.21[c]	96.36±0.25[a]	44.16±1.08[c]	45.52±0.36[d]	15.92±0.69[a]
Jowar	28.14±2.13[c]	93.99±0.09[c]	44.61±1.90[c]	46.82±0.88[d]	15.37±1.11[a]
Wheat	70.40±2.74[a]	95.05±0.19[b]	83.41±2.44[a]	63.72±0.90[a]	6.34±0.30[d]
Ragi	31.04±1.23[c]	87.86±0.36[f]	47.63±1.10[c]	50.66[c]±1.4[b]	12.94±0.49[b]
Barley	70.07±0.29[a]	91.55±0.37[e]	81.38±0.26[a]	62.33±0.65[a]	5.99±0.02[d]
Bajra	50.07±0.17[b]	92.53±0.19[d]	64.63±0.15[b]	58.56±0.69[b]	8.46±0.01[c]
SEM	4.112	0.575	3.710	1.742	0.928
P Value	0.001	0.001	0.001	0.001	0.001

Each value is an average of three observations
[abcd]Means with different superscripts in a column differ significantly: P<0.01
IVGP-in vitro gas production, IVDMD-in vitro dry matter degradability, IVOMD-in vitro organic matter degradability, IVNDFD-in vitro neutral detergent fibre degradability, PF- Partitioning Factor
SEM: Standard Error Mean; P: Probability value

Broken rice showed highest (P<0.01) IVDMD% i.e., 96.36 followed by wheat (95.05). The IVDMD % for jowar and maize was and, and was comparable, but lower than broken rice and wheat. The IVDMD in other cereal grains, decreased linearly in order (P<0.01) for bajra, barley and ragi. The highest and lowest IVDMD of broken rice and ragi grains was probably due to presence of lowest (%) and highest (%) crude fibre content, respectively observed (Table 1). The higher degradability of wheat was due to presence of floury endosperm. The lower degradability in maize and jowar was probably due to presence of hard seed coat (Rooney and Pflugfelder, 1986). The results obtained in this study are in accordance with values obtained by earlier researchers like Yang *et al.* (2018), who evaluated three total mixed rations containing rice, corn and wheat in each one and reported that rice TMR with higher degradability than the corn and wheat TMRs.Yoo *et al.* (2020) reported that the IVDMD of rice TMR was higher than that of corn TMR (P<0.05). Allister *et al.* (2020) evaluated the ISDMD (In sacco DM disappearance) of barley, maize, sorghum and wheat and reported wheat exhibited the highest ISDMD followed by barley, sorghum and maize, respectively corroborating with the present findings. Rosendo *et al.* (2013) reported that IVTDMD (%) was higher for maize than sorghum grain after 48 hours of the incubation, while in our study, no difference between maize and sorghum grain was observed, which could be due to less incubation time of 24 h in present study.

While the IVOMD % was highest (P<0.01) in wheat and barley followed by bajra in comparison to other cereals (Table 2). The IVOMD % in rest of the cereals viz., maize, broken rice, jowar and ragi was comparable among each other.The main component in cereal grains is (60-80%) starch which is highly degradable compared to fibre, but the proteinaceous matrix surrounding starch granules affects the starch digestibility. Digestibility is negatively associated with the presence of prolamins which are the storage proteins. The prolamins in maize, sorghum, wheat, barley and rice are zein, kafirins, gliadin, hordeins and secalins, respectively and their concentration varies among the cereals. Usually, wheat, rice and barley have fewer prolamins than corn, bajra and sorghum (Momany *et al.,* 2006 and Giuberti *et al., 2*014).The starch source is also an important factor that affects the degradability of cereal grains. Corn and especially sorghum have a high proportion of peripheral and horny endosperm resulting in increased resistance to microbial activity (Rooney and Pflugfelder, 1986), unlike wheat, which have higher proportion of floury endosperm. In addition, corn and sorghum have a denser protein matrix (Kotarski *et al.,* 1992). The in vitro experiment by Lanzas *et al.* (2007) measured fractional gas rates, as a measure of starch digestion (Huhtanen and Sveinbjornsson, 2006), reporting 0.26, 0.24, 0.15, and 0.06 h-1 rates for wheat, barley, corn and sorghum, respectively.

The higher (P<0.01) IVOMD observed in wheat and barley in the present study was could be due to the presence of fewer prolamins than the other cereal grains (Momany *et al.,* 2006; Giuberti *et al.,* 2014). The lowest IVOMD of corn and sorghum grain was due to higher prolamins and higher proportion of peripheral and horny endosperm which resulted in increased resistance to microbial activity. The lowest IVDMD and IVOMD of ragi grain might be due to presence of higher crude fibre (Table 1) content.

The IVNDFD % ranged from 44.38 to 63.72 with significant (P<0.01) difference among the cereal grains (Table 2). The IVNDFD (%) was highest in wheatand barley followed by bajra and then inragi and the lowest IVNDFD was observed in jowar, broken rice and maize. The IVNDFD % in latter 3 cereals was comparable. The IVNDFD of cereal grains in this study followed the trend of IVOMD except for ragi grain. The lower IVNDFD of maize, jowar and broken rice was could be attributed to their increased resistance to microbial activity because of their denser protein matrix and higher peripheral and horny endosperm in the grain (Rooney and Pflugfelder, 1986). In disagreement with present study reports, Yoo *et al.* (2020) reported that IVNDFD of rice TMR was higher than corn TMR which could be the result of associative effect of other feed ingredients in TMR. Yang *et al.*(2018) reported that lower IVNDFD was observed with wheat TMR than with corn TMR and rice TMR.

Significant (P<0.01) variation was also observed for partitioning factor (PF) among the cereals. The higher PF was noticed for broken rice, jowar and maize followed by ragi and then by bajra and lowest in wheatand barleyand was in accordance with efficiency of microbial biomass production observed for various cereal grains (Table 3).

Table 3. Microbial biomass production, efficiency of microbial biomass production and metaboliz able energy of cereal grains assessed by in vitro gas production

Cereal grain	MBP (mg/500mg)	EMBP (g/kg DOM)	ME (MJ/kg)
Maize	138.33±1.04[c]	690.08±2.70[ab]	10.27±0.02[e]
Broken Rice	140.47±2.17[c]	695.42±6.03[a]	10.98±0.16[d]
Jowar	137.12±4.65[c]	689.82±9.04[ab]	10.33±0.29[e]
Wheat	230.23±14.37[a]	597.11±13.15[d]	18.04±0.37[a]
Ragi	140.06±1.75[c]	672.53±5.99[b]	9.66±0.17[f]
Barley	215.00±0.39[a]	582.41±0.83[d]	16.21±0.04[b]
Bajra	180.52±0.60[b]	621.04±0.66[c]	14.16±0.02[c]
SEM	8.486	10.225	0.692
P Value	0.001	0.001	0.001

Each value is an average of three observations

[abc]Means with different superscripts in a column differ significantly: P< 0.01

MBP (mg/500 mg) - Microbial biomass production, EMBP (g/kg DOM) - Efficiency of microbial biomass production, ME (MJ/Kg) - Metabolizable energy

SEM: Standard Error Mean; P: Probability value

The MBP (mg/500mg) ranged from 137.12 (jowar) to 230.38 (wheat) with significant ($P<0.01$) difference among the cereals (Table 3). The highest MBP (mg/500mg) was noticed for wheat and barley followed by bajra and lowest in maize, broken rice, ragi and jowar. While, the mean EMBP (g/kg DOM) values ranged from 582.41 (barley) to 695.42 (broken rice) showing significant ($P<0.01$) difference among the cereals. The highest EMBP (g/kg DOM) wasfor broken rice and comparable values were observed among broken rice, maize and jowarfollowed by ragi and then by bajra and lowest EMBP was in barley and wheat.The EMBP calculated from the ratio of MBP (mg) to IVOMD (mg) indicating its negative correlation with the IVOMD (mg). The lower EMPB of wheat and barley grains than the other cereal grains noticed was due to their higher IVOMD values. There will be inverse relationship between in vitro gas production and microbial biomass yield (Blummel *et al.,* 1996). This is in corroboration with the present study findings (Table 2 and 3).

The ME (MJ/kg) contentin these cereal grains significantly ($P<0.01$) differed ranging from 9.66 (ragi) to 18.04 (wheat). The descending order of the cereal grains according to their ME (MJ/kg) values was wheat > barley >bajra> broken rice >jowar and maize >ragi.The ME content of cereal grains positively correlated with the CP content in grains and gas volume produced (Table 1 and 3).

The mean pH values ranged from 7.00 to 7.23 and were statistically significant ($P<0.01$) and presented in Table 4.

Table 4. Ruminal pH, total volatile fatty acid and ammonia nitrogen concentration of various cereal grains assessed by in vitro gas production technique

Cereal grain	pH	TVFA(mmol/dl)	NH3-N(mg/40ml)
Maize	7.23±0.03^{a}	3.72±0.16^{d}	17.92±0.01
Broken Rice	7.20±0.01^{ab}	3.45±0.05^{d}	17.17±0.75
Jowar	7.23±0.03^{a}	3.85±0.21^{cd}	19.41±0.75
Wheat	7.00±0.01^{c}	6.50±0.09^{a}	15.68±0.01
Ragi	7.13±0.03^{b}	3.70±0.03^{d}	16.43±1.49
Barley	7.00±0.01^{c}	5.78±0.16^{b}	15.68±0.01
Bajra	7.17±0.03^{ab}	4.17±0.11^{c}	17.17±1.49
SEM	0.022	0.249	0.393
P Value	0.001	0.001	0.11

Each value is an average of three observations

^{abc}Means with different superscripts in a column differ significantly: P=0.001

TVFA- Total volatile fatty acid, NH3-N- Ammonia nitrogen

SEM: Standard Error Mean; P: Probability value

The pH was highest from maizeand jowar followed by broken rice and bajra and were comparable to maize, jowar and ragi. The pH in ragi was lower than maize and jowar but higher than wheat and barley. The pH in wheat and barley was lowest. The pH value is negatively correlated with TVFA production and positively correlated with NH3-N. The lowest pH in wheat is due to the highest TVFA and highest pH in maize and broken rice is due to the lowest production of TVFA. According to the findings of present study results, Amanzougarene *et al.* (2020) reported highest ($P<0.05$) pH in sorghum and maizethan barley.Opatpatanatik *et al.* (1994) reported that pH was lower ($p<0.05$) with time for wheat than maize grain. Yoo *et al.* (2020) reported that ruminal pH was higher in corn TMR ($p<0.05$) than in rice TMR. Yang *et al.* (2018) reported that rice TMR maintained lowest pH value than the wheat and corn TMRs. While, Yang *et al.* (2020) reported that pH was not affected (P>0.10) with substitution of rice in the TMR instead of corn TMR which is in agreement with the present study findings.

The TVFA (mmol/dl) concentration differed significantly (P<0.01) among the cereal grains, being highest in wheat, followed by barley and then in bajra and jowar. The TVFA in jowar and bajra was comparable and lowest TVFA was observed in maize, ragi and broken rice. This result was in consistent with the results of Opatpatanatik et al. (1994) who reported that total VFA production from wheat was higher than the maize grain due to the higher (P<0.05)starch degradability of wheat.Yang *et al.* (2018) reported that rice TMR with lower VFA concentration than the wheat TMR (P<0.05).In disagreement with the present study reports, Yang *et al.* (2020) and Yoo *et al.* (2020) reported TVFA production from rice TMR was higher (P<0.05) than corn TMR.

In this study, maize, broken rice and sorghum fermented more slowly. The protein matrix was probably the major factor responsible for limiting the access of rumen microbes to starch granules of maize and sorghum (Rooney and Pflugfelder 1986 and McAllister *et al.,* 1993). The composition and kernel structure of sorghum and maize are similar and they have much higher proportion of peripheral endosperm which is extremely dense, hard and resistant to water penetration and digestion (Rooney and Pflugfelder., 1986) and in addition sorghum also contain tannins that could reduce starch digestion. These possible reasons could have resulted in lower starch degradability and lead to lower TVFA production from these grains.

The mean NH3-N (mg/40 ml) ranged from 15.68 to 19.41 and was comparable among various cereal grains.This is in accordance with the results of Yang *et al.* (2020) and Yoo *et al.* (2020) reported that NH3-N was not affected (P>0.10) with substitution of rice in the TMR instead of corn TMR. The present study results are in disagreement with the results of Yang *et al.* (2018), who noticed highest NH3-N concentration for wheat based TMR than the corn and rice based ones.

CONCLUSION

The in vitro degradability and fermentation of maize, broken rice, ragi and sorghum grains are much slower than that of wheat, barley and bajra. The rumen degradability and rumen fermentation pattern of broken rice was comparable to maize grain.

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