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In vitro Evaluation of Concentrate Mixtures containing Graded Levels of Malt Sprouts

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ABSTRACT

The present study was conducted to assess the chemical composition and *in vitro* nutritional worth of concentrate mixtures containing graded levels of malt sprouts. Malt sprouts in the concentrate feed was included at 0, 10, 20, 30 and 40% levels to make five isonitrogenous concentrate mixtures. Crude protein content of concentrate mixtures varied from 19.61% to 20.32%. Ether extract content in concentrate mixtures varied from 5.33% to 5.96%. No significant difference was observed in truly degraded substrate, partitioning factor, OM digestibility, NDF digestibility, DM digestibility and ammoniacal nitrogen among the concentrate mixtures tested. However, the net gas production, short chain fatty acids and metabolizable energy were lower ($P<0.05$) in concentrate mixture 5 (containing 40% malt sprouts) than other concentrates evaluated. It was concluded that malt sprouts could be could be incorporated upto 30% in the concentrate mixture of ruminants without affecting nutrient digestibility, ME availability and propionate production.

HIGHLIGHTS

- The level of malt sprouts had no adverse effect on nutrient digestibility of concentrates evaluated.
- The net gas production, short chain fatty acids and metabolizable energy were lower ($P<0.05$) in conc 5 containing 40% malt sprouts.

Keywords: *In vitro* evaluation, Malt sprouts, Volatile fatty acids

India has a large livestock population. The livestock sector accounts for 4.11% of overall GDP and 25.6% of total agriculture GDP. The total livestock population in the country was 535.78 million (20th livestock census), representing a 4.6 percent increase over the previous Livestock Census in 2012. The goat population is 148.88 million among the total livestock population. Traditionally goat milk has been known for its medicinal properties since ancient times. It has recently gained importance in human health due to its proximity to human milk for easy digestibility due to its smaller fat globules and its health-promoting traits. It has a crucial role in providing extra incomes and livelihoods to India's poor and rural landless farmers. Due to the rise in human consumption of cereal grains, the supplementing cost of cereals to animals increases drastically. Agro industrial by-products are supplemented with roughage-based diets as alternative feed resources. Presently, the use of agro-industrial by-products is becoming popular among livestock owners as they are relatively cheap when compared with the conventional ingredients used in feed preparation (Nurfeta, 2010). Malt sprout is a by-product of malt processing companies. It is the dried roots and shoots left after the extraction of malt from germinated cereal. Malting leads to an increase in the nutritive value of grains. Moreover, various anti-nutritional factors are eliminated during germination (Girma and Gebremariam, 2018). Therefore, keeping in

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view the above points, a comprehensive study was taken up to evaluate an alternate feedstuff malt sprouts in the diet of ruminants by *in vitro* gas production technique.

MATERIALS AND METHODS

Chemical analysis

The concentrate mixtures containing graded levels of malt sprouts i.e. 10, 20, 30 and 40% were prepared (Table 1) and analysed for proximate (AOAC, 2005) and cell wall constituents (Van Soest *et al*., 1991).

In vitro evaluation

Male buffaloes fitted with rumen fistulae maintained on 2 kg conventional concentrate mixture (maize-38, mustard cake-15, SBM- 15, deoiled rice bran-12, wheat bran-10, rice polish- 7, mineral mixture-2, common salt-1part), 17 kg green fodder, 3 kg wheat straw and *ad lib* urea molasses mineral block were used as a donor for rumen liquor. The rumen contents were collected and then strained through 4 layered muslin cloth. The strained rumen liquor (SRL) was added to the buffer media (containing macro, micro mineral solutions, resazurin and a bicarbonate buffer solution prepared as per (Menke *et al*., 1979; Menke and Steingass, 1988) in 1:2 ratio. The medium was kept at 39ºC in a water bath and flushed with CO_2. Thirty ml of buffered rumen fluid was dispensed into 100 ml calibrated glass syringes containing 375 mg test feed under the anaerobic conditions. Syringes were sealed with rubber tube and plastic clip and placed in a water bath at 39ºC for 24 h. Blank was also run in triplicate with each set which only contained buffered rumen liquor. After 24 h, the volume produced in each syringe was recorded and the contents of syringes were transferred to spoutless beaker, boiled with neutral detergent solution for estimating the OM and NDF digestibility (Van Soest and Robertson, 1988). The amount of gas produced was used to calculate ME. The partitioning factor (PF) was calculated as per the method described by France *et al*. (1993).

Estimation of volatile fatty acids

Volatile fatty acids were estimated using Netchrom 9100 gas chromatograph (Netel, New Delhi, India) equipped with flame ionization detector as per method described by Cottyn and Boucque (1968). The gas column (6 ft length and 1/8 inch diameter) packed with chromosorb 101 was used for the estimation of VFA. The gas flows for nitrogen, hydrogen and zero air were 15, 30, and 300 ml/min, respectively. Temperature of injector oven, column and detector were 250°C, 175°C and 270ºC respectively. Samples were prepared by adding 0.2 ml of 25% metaphosphoric acid per ml of rumen liquor/contents of *in vitro* syringes, allowing it to stand for 2 h followed by centrifugation at 4000 rpm for 7 min. Supernatant was used for estimation of VFA. Standard VFA mixture was prepared by mixing stock solutions (each of 25 mg/ml concentration) of standard VFAs and distilled water in the proportion of acetic acid 1.68 ml, propionic acid 0.48 ml, isobutyric acid 0.12 ml, butyric acid 0.24 ml, isovelaric acid 0.12 ml, valeric acid 0.12 ml and make the volume to 10 ml to obtain final concentration of acetic acid, 7.0, propionic acid, 1.62; isobutyric acid, 0.34; butyric acid 0.68; isovaleric acid 0.29 and valeric acid 0.29 mM/100 ml. The standard was stored in deep freeze until further use.

Determination of ME availability

The ME value of the substrate was calculated by using the following equation developed by Menke *et al* (1979).

$$ME\ (kg) = 1.24 + 0.146\ G\ (ml/200\ mg\ DM) + 0.007\ CP + 0.0244\ EE$$

where,

ME = Metabolizable energy, MJ/kg DM

G =Net gas production, ml/200mg DM

CP = Crude protein, g/ kg

EE = Ether extract, g/kg.

Determination of hydrogen recovery

Hydrogen recovery (%) was estimated as (4M+2P+2B) / (2A+P+4B) × 100, the ratio of hydrogen consumed via CH_4/VFA was estimated as 4M/(2P+2B), where acetate (A), propionate (P), butyrate (B) and methane (M) production was expressed in mmol by Demeyer (1991).

Table 1: Ingredient composition of concentrate mixtures (parts/100 parts)

Ingredient	CONC 1 (0% MS)	CONC 2 (10% MS)	CONC 3 (20% MS)	CONC 4 (30% MS)	CONC 5 (40% MS)
Maize	40.00	40.00	40.00	40.00	38.00
Soybean	22.00	20.00	18.00	16.00	14.00
Wheat bran	20.00	15.00	10.00	5.00	0.00
Rice polish	5.00	5.00	5.00	5.00	5.00
Deoiled rice bran	10.00	7.00	4.00	1.00	0.00
Mineral mixture	2.00	2.00	2.00	2.00	2.00
Malt sprouts	0.00	10.00	20.00	30.00	40.00
Salt	1.00	1.00	1.00	1.00	1.00

Determination of fermentation efficiency

This was calculated on the basis of the equation worked out by Orskov (1975) and modified by Baran and Zitnan (2002)

$$FE = (0.622a + 1.092p + 1.56b)\ 100/\ (a+p+2b)$$

Where: a, p, and b express the concentration (µmol) of acetic, propionic and butyric acids respectively in the total concentration of VFA produced. The final results of this equation have been expressed in percentage and show an amount of energy stored in VFAs as a percentage participation of the initial energy.

Determination of VFAs utilization index

This was expressed by non-glucogenic VFAs/glucogenic VFAs ratio (NGGR) according to Orskov (1975).

$$NGGR = (A + 2B + V) / (P+V)$$

Where A, P, B and V express the concentrations (µmol) of acetic, propionic, butyric, and valeric acids, respectively. Valeric acid is classified as both glucogenic and non-glucogenic VFA because its oxidation creates 1 mole of acetic acid and 1 mole of the propionic acid. Too high NGGR indicates high loss of energy in the form of gases.

STATISTICAL ANALYSIS

Data were analysed by simple ANOVA, as described by Snedecor and Cochran (1994), by using SPSS (2012) version 21. The differences in means were tested by Tukey's b.

RESULTS AND DISCUSSION

Chemical composition of concentrate mixtures containing graded levels of CSM

The chemical composition of various concentrates with graded level of malt sprouts is given in Table 2. The OM content of concentrate mixtures varied from 90.82% to 92.15%. All the concentrates mixtures prepared were isonitrogenous. The CP content of concentrate mixtures was in the range of 19.61% to 20.32% (Table 2). The ether extract content in concentrate mixture having 0% malt sprouts level (control) was 5.76% while for concentrate mixtures having graded levels of malt sprouts, it ranged between 5.33% to 5.96%. The total ash content in concentrate mixtures varied from 7.85% to 9.17%. An increasing trend was seen in total ash content by inclusion of malt sprouts in the concentrate mixtures.

NDF content in concentrate mixtures ranged between 38.50% to 39.90% (Table 2). ADF content in concentrate mixture 1 was 19.50% while from concentrate 2 to 5 it ranged between 15.55% to 16.75%. Hemicellulose content of concentrate 1, concentrate 2, concentrate 3, concentrate 4 and concentrate 5 was 19.30, 23.45, 23.90, 21.75 and 23.70%, respectively. ADL content of concentrates ranged between 4.70% to 6.20%. ADICP content in concentrate mixtures was 5.83, 5.63, 6.02, 6.02 and 6.22% for concentrate 1, concentrate 2, concentrate 3, concentrate 4 and concentrate 5, respectively. NDICP content ranged between 9.13% to 11.85%. The total carbohydrate content in concentrates ranged between 66.12% to 66.41%.

Table 2: Chemical composition of concentrate mixtures containing graded levels of malt sprouts, % DM basis

Parameter	CONC 1 (0% MS)	CONC 2 (10% MS)	CONC 3 (20% MS)	CONC 4 (30% MS)	CONC 5 (40% MS)
OM	91.60	92.15	91.99	90.82	91.00
CP	19.72	19.93	19.95	19.61	20.32
EE	5.76	5.92	5.63	5.96	5.33
Total ash	8.40	7.85	8.01	9.17	9.00
NDF	38.80	39.00	39.90	38.50	39.50
ADF	19.50	15.55	16.00	16.75	15.80
Hemicellulose	19.30	23.45	23.90	21.75	23.70
ADL	4.80	6.20	5.20	5.55	4.70
ADICP	5.83	5.63	6.02	6.02	6.22
NDICP	9.13	9.33	10.68	11.85	11.46
TCHO	66.12	66.30	66.41	65.26	65.35

OM- Organic matter, CP- Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, ADL- Acid detergent lignin, TCHO- Total carbohydrates, ADICP- Acid detergent insoluble crude protein, NDICP- Neutral detergent insoluble crude protein.

Table 3: *In vitro* utilization of nutrients of concentrate mixtures containing graded levels of malt sprouts (24 h)

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
NGP, ml/g DM/24h	183.55^{b}	190.22^{b}	181.55^{b}	188.89^{b}	158.22^{a}	4.18
TDS, mg	343.50	345.56	344.96	340.59	341.25	0.66
PF, mg/ml	4.43	3.81	4.04	3.91	4.65	0.15
OMD, %	78.60	78.44	79.71	81.36	80.81	0.52
NDFD, %	49.48	49.06	53.22	56.02	55.78	1.29
MMP, mg	114.17^{a}	114.13^{a}	125.18^{ab}	121.26^{ab}	145.22^{b}	4.18
EMMP, %	42.28^{a}	42.12^{a}	45.53^{ab}	43.75^{a}	52.63^{b}	1.39
DMD, %	79.47	78.40	80.27	80.93	81.20	0.44
SCFA, mmole	0.83^{b}	0.84^{b}	0.80^{b}	0.83^{b}	0.70^{a}	0.02
ME, MJ/kg DM	9.12^{b}	9.43^{b}	9.12^{b}	9.12^{b}	8.18^{a}	0.12
NH_3-N, mg/dl	39.00	35.50	36.50	35.50	35.00	0.58
Fer CO_2, mmol	48.06^{a}	49.05^{ab}	50.40^{abc}	49.65^{bc}	51.64^{c}	0.43
Fer CH_4, mmol	25.26^{a}	27.02^{ab}	28.55^{b}	27.99^{b}	31.30^{c}	0.68

NGP- Net gas production, TDS- Truly degraded substrate, PF- partitioning factor, D- digestibility, OM- organic matter, NDF- neutral detergent fibre, MMP- microbial mass production, EMMP- efficiency of microbial mass production, DM- dry matter, SCFA- short chain fatty acids, NH_3-N-ammoniacal nitrogen, Fer CO_2- fermentable carbon dioxide, Fer CH_4- fermentable methane, Means bearing different superscripts in a row differ significantly (P<0.05).

In vitro evaluation of concentrate mixtures containing graded levels of malt sprouts

The net gas production (NGP, ml/g DM/24 h) in concentrate mixture 5 (158.22) was lower (P<0.05) than concentrate mixture 1 (183.55), concentrate mixture 2 (190.22), concentrate mixture 3 (181.55) and concentrate mixture 4 (188.89) (Table 3). Our results are in accordance with those of Saka *et al*. (2018) who reported significant difference in gas production in diets which contained graded levels of malted sorghum sprout mixed with pineapple waste (MSPW) (0%, 20%, 40% and 60%) in which 60% MSPW recorded the highest (P<0.05) gas production value (15.552 ml/200 mgDM).

The truly degraded substrate varied non significantly among the concentrate mixtures. The truly degraded substrate (TDS, mg) was 343.50, 345.56, 344.96, 340.59 and 341.25 in concentrate mixtures 1, 2, 3, 4 and 5, respectively (Table 3). The partitioning factor (PF, mg/ml) was 4.43, 3.81, 4.04, 3.91 and 4.65 in concentrate mixtures 1, 2, 3, 4 and 5, respectively. No significant difference

was observed in PF among the concentrate mixtures. The partitioning factor (PF) is the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (ml) produced. A higher partitioning factor means proportionally more of degraded matter is incorporated into microbial mass i.e. the efficiency of microbial protein synthesis is higher.

The OM digestibility (%) in concentrate mixture 1, 2, 3, 4 and 5 was 78.60, 78.44, 79.71, 81.36 and 80.81, respectively and varied non-significantly (Table 3). Our results are contrary to those of Saka *et al*. (2018) who reported that organic matter digestibility varied significantly (P<0.05) across the dietary treatment with diet containing 20% malted sorghum sprout mixed with pineapple waste (MSPW) giving the highest (P<0.05) value (62.89%) when MSPW was added in graded levels (0%, 20%, 40% and 60%) in the diets of goats. The NDF digestibility did not vary significantly among the concentrate mixtures.

The efficiency of microbial mass production (EMMP,%) was similar in concentrate mixture 1 (42.28), 2 (42.12) and 4 (43.75) with highest (P<0.05) value in concentrate 5 (52.63). The DM digestibility of concentrate mixtures varied non significantly. The short chain fatty acids (SCFA, mmole) production was highest (P<0.05) in concentrate mixtures 1 (0.83), 2 (0.84), 3 (0.80) and 4 (0.83) while it was lowest (P<0.05) in concentrate mixture 5 (0.70). The results of the present study are in agreement with those of Saka *et al*. (2018) who reported significant difference in SCFA production when malted sorghum sprout mixed with pineapple waste (MSPW) was fed in graded levels (0%, 20%, 40% and 60%) in the diet of goats. The metabolizable energy (ME, MJ/kg DM) was lowest (P<0.05) in concentrate mixture 5 (8.18), however, it was similar among other concentrate mixtures and ranged between 9.12 to 9.43 (Table 4). Similar to the present study, Saka *et al*. (2018) also reported significant difference in ME of diets which contained graded levels of MSPW (0%, 20%, 40% and 60%) and the diet containing 20% MSPW recorded the highest (P<0.05) metabolizable energy (9.54 MJ/kg) in goats.

The ammonia nitrogen (NH_3-N, mg/dl) varied non-significantly among concentrate mixtures tested and the values varied from 35.00 to 39.00. However, Saka *et al*. (2018) reported that ammoniacal nitrogen was significantly higher (P<0.05) with inclusion level of 20% malted sorghum sprout mixed with pineapple waste (MSPW) when added at the rate of 0, 20, 40 and 60% in the diet of goats. The fermentable carbon dioxide (Fer CO_2, mmol) was highest (P<0.05) in concentrate mixture 5 (51.64) followed by concentrate mixture 3 (50.40), 4 (49.65) and 2 (49.05) while it was lowest (P<0.05) in concentrate mixture 1 (48.06). Similarly, the fermentable methane (Fer CH_4, mmol) was higher (P<0.05) in concentrate mixture 5 (31.30) while it was lower (P<0.05) in concentrate mixture 1 (25.26) than other concentrate mixtures tested. The

Table 4: *In vitro* volatile fatty acids production (mM/dl) in concentrate mixtures containing graded levels of malt sprouts (24 h)

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
Acetate	2.46^{a}	2.58^{ab}	2.73^{c}	2.73^{c}	2.70^{bc}	0.04
Propionate	1.29^{b}	1.21^{b}	1.17^{b}	1.21^{b}	0.95^{a}	0.04
Isobutyrate	0.00	0.00	0.00	0.00	0.00	0.00
Butyrate	0.32	0.34	0.39	0.36	0.37	0.01
Isovalerate	0.16	0.15	0.15	0.14	0.12	0.01
Valerate	0.00	0.00	0.00	0.00	0.00	0.00
TVFA	4.23	4.27	4.44	4.44	4.14	0.05
A:P	1.91^{a}	2.13^{ab}	2.34^{b}	2.27^{b}	2.84^{c}	0.11
Relative proportion, %						
Acetate	58.20^{a}	60.37^{ab}	61.53^{b}	61.51^{b}	65.23^{c}	0.78
Propionate	30.48^{c}	28.37^{bc}	26.27^{b}	27.20^{b}	22.96^{a}	0.85
Isobutyrate	0.00	0.00	0.00	0.00	0.00	0.00
Butyrate	7.56	7.85	8.71	8.06	8.86	0.19
Isovalerate	3.76	3.41	3.49	3.23	2.95	0.12
Valerate	0.00	0.00	0.00	0.00	0.00	0.00

TVFA- Total volatile fatty acids, A:P- acetate: propionate, Means bearing different superscripts in a row differ significantly (P<0.05).

Table 5: Hydrogen balance of nutrients of concentrate mixtures containing graded levels of malt sprouts (24 h)

Parameter	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
H- recovery, %	103.09	102.71	100.34	100.48	105.11	0.69
H- consumed via CH_4	3.55	3.59	3.75	3.73	3.39	0.05
FE, %	78.30^{c}	77.34^{bc}	76.55^{b}	76.83^{b}	75.03^{a}	0.37
VFA UI	2.41^{a}	2.68^{ab}	3.01^{ab}	2.86^{b}	3.61^{c}	0.14

FE- fermentation efficiency, H- Hydrogen, VFA UI- volatile fatty acids utilization index, Means bearing different superscripts in a row differ significantly (P<0.05).

fermentable methane (Fer CH_4, mmol) was 27.02, 28.55 and 27.99 in concentrate mixture 2, 3 and 4, respectively.

The acetic acid content (mM/dl) was lowest (P<0.05) in concentrate mixture 1 (2.46) while concentrate mixture 3 (2.73) and 4 (2.73) produced highest (P<0.05) acetic acid followed by concentrate mixture 5 (2.70) and concentrate mixture 2 (2.58) (Table 4). The propionic acid content (mM/dl) was lowest (P<0.05) in concentrate mixture 5 (0.95) while it was highest (P<0.05) in concentrate mixtures 1 (1.29), 2 (1.21), 3 (1.17) and 4 (1.21). There was non-significant difference in butyric acid, isovaleric acid and total volatile fatty acid production among the concentrate mixtures. The acetate: propionate (A: P) ratio was highest (P<0.05) in concentrate mixture 5 (2.84) followed by concentrate mixture 3 (2.34), concentrate mixture 4 (2.27) and concentrate mixture 2 (2.13) whereas it was lowest (P<0.05) in concentrate mixture 1 (1.91).

The relative proportion (%) of acetic acid was highest (P<0.05) in concentrate mixture 5 (65.23) while it was lowest (P<0.05) in concentrate mixture 1 (58.20) with intermediate values in concentrate mixture 2 (60.37), concentrate mixture 3 (61.53) and concentrate mixture 4 (61.51) (Table 4). The relative proportion of propionic acid (%) was highest (P<0.05) in concentrate mixture 1 (30.48) followed by concentrate mixture 2 (28.37), concentrate mixture 3 (26.27), concentrate mixture 4 (27.20) while it was lowest (P<0.05) in concentrate mixture 5 (22.96). There was non-significant difference in butyric and isovaleric acid content among the concentrate mixtures. The relative proportion of butyrate and isovalerate ranged between 7.56 to 8.86% and 2.95 to 3.76%, respectively.

The hydrogen recovery (%) was 103.09, 102.71, 100.34, 100.48 and 105.11 in concentrate mixture 1, 2, 3, 4 and 5, respectively (Table 5). There was non-significant difference in hydrogen recovery and hydrogen consumed via CH_4 among the concentrate mixture tested. The hydrogen consumed via CH_4 ranged between 3.39 to 3.75. The fermentation efficiency (%) was highest (P<0.05) in concentrate mixture 1 (78.30) and was lowest (P<0.05) in concentrate mixture 5 (75.03). The fermentation efficiency (%) was 77.34, 76.55 and 76.83 in concentrate mixture 2, 3 and 4, respectively. The volatile fatty acids utilization index (VFA UI) or non- glucogenic to glucogenic VFA ratio was lowest (P<0.05) in concentrate mixture 1 (2.41) and highest (P<0.05) in concentrate mixture 5 (3.61). The lowest (P<0.05) VFA UI in concentrate 1 correlates well with the highest (P<0.05) molar proportion of propionate in it. The volatile fatty acids utilization index (VFA UI) in concentrate mixture 2, 3 and 4 was 2.68, 3.01 and 2.86, respectively.

CONCLUSION

Nutrient digestibility (OM, NDF, DM) and ammoniacal nitrogen were similar among the concentrates evaluated, indicating that level of malt sprouts had no adverse effect on digestibility. However, the net gas production, short chain fatty acids and metabolizable energy were lower (P<0.05) in concentrate mixture 5 (containing 40% malt sprouts) than other concentrates evaluated. The data conclusively revealed that malt sprouts could be could be incorporated upto 30% in the concentrate mixture without affecting nutrient digestibility, ME availability and propionate production.

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