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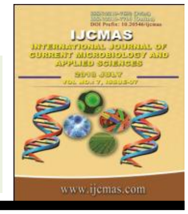

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In vitro Nutrient Digestibility and Fermentation Pattern of Concentrate Mixtures Containing Incremental Levels of Dried Distillers Grains with Solubles

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A B S T R A C T

The main objective of the present study was to investigate the effect of incorporation of graded levels of RDDGS in the concentrate mixtures on *in vitro* nutrient digestibility and rumen fermentation pattern using buffalo inoculum. Soybean meal (SBM) based conventional concentrate mixture was prepared and SBM in the concentrate mixture was replaced by RDDG at graded levels of 25, 50, 75 and 100 per cent on N basis. The nutritional worth of various concentrate mixtures was assessed by *in vitro* gas production technique. The *in vitro* study of concentrates containing graded levels of RDDGS revealed that OM, NDF and DM digestibility increased (P<0.05) with the increasing level of RDDGS in the concentrate mixture. The MMP of concentrate mixtures increased (P>0.05) with increasing levels of RDDGS replacing soybean meal. However, the increase in MMP was statistically non-significant. The ME availability was highest (P<0.05) in concentrate mixtures containing 50 and 75% RDDGS replacing soybean meal. The ammonical-N of the concentrate mixtures decreased (P<0.05) with the increase in the inclusion of graded levels of RDDGS in concentrate mixtures replacing SBM. The acetic acid content and A:P (mM/dl) ratio decreased(P<0.05) with increasing level of RDDGS replacing SBM in the concentrate mixtures. The inclusion of graded levels of RDDGS in the concentrate mixture was observed to have no significant effect on the methane production in concentrate mixtures. Hence, it was concluded that RDDGS could be considered as promising protein supplement for livestock and can replace conventional oilseed cakes *viz*., soybean meal upto 75 % in the concentrate mixture without any adverse effect on nutrient digestibility, ME availability, fermentation efficiency and methane production.

Keywords

Buffalo inoculum, Dried distillers grains with solubles, *In vitro* digestibility, Hydrogen balance, Methane

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Introduction

In developing countries like India, livestock and dairy plays an important role in the economy and livelihood of people. Livestock contributes 25.6% of total value of output in agriculture which is 4.11% of total GDP (National livestock census, 2012). In India, due to its tropical climatic conditions, urbanization, water scarcity, industrialization and non-availability of cultivable land, the gap between availability and requirement of nutrients and feedstuffs is increasing. Around 60-70 % of the cost of production is involved in feeding of animals. The cost of conventional feed resources used for feeding livestock has increased because of increased needs of grains by the human population.

Dried distillers grains solubles (DDGS) is an agro-industrial by-product obtained as a co-product in the production of bio-ethanol from various grains like maize, sorghum, wheat, rice and barley etc. Rice dried distillers grains solubles (RDDGS) is the major co-product from alcohol and rice wine production using broken rice in Asian countries. Very less information is available on the use of RDDGS as a substitute of costly conventional protein sources in livestock ration. The present work examines the effect of replacement of soybean meal with rice dried distillers grains with solubles in the concentrate mixtures at graded levels on *in vitro* nutrient digestibility, methane production and rumen fermentation parameters in buffalo inoculum.

Materials and Methods

Sample collection and preparation

Conventional concentrate mixture was prepared (maize 34, SBM 15, mustard cake15, wheat bran 10, rice polish 6, deoiled rice polish 17, mineral mixture 2 and salt 1 part). Soybean meal in the concentrate mixture was replaced by RDDGS at 0, 25, 50, 75 and 100% levels on N basis to formulate 5 concentrate mixtures.

Proximate and cell wall constituents

Concentrate mixtures were analyzed for dry matter (DM), Kjeldahl N, ether extract (EE) and ash content using the standard procedures (AOAC, 2000). Crude protein (CP) content of samples was determined as Kjeldahl N × 6.25 by digesting in sulphuric acid and digestion mixture (consisting of sodium/potassium sulphate and copper sulphate in10:1 ratio) using semiauto-analyser (Kel Plus Classic-DX, Pelican). Cell wall fractions, *viz.* NDF, ADF, cellulose and lignin were estimated sequentially using the standard procedure (Van Soest *et al.,* 1991). NDF and ADF were expressed inclusive of residual ash. Lignin was determined by solublization of cellulose with72 per cent sulphuric acid. Acid detergent insoluble protein and neutral detergent insoluble protein were determined as per Licitra *et al.,* (1996).

In vitro evaluation

The nutritional worth of various concentrates formulated was assessed by *in vitro* gas production technique (Menke *et al.,* 1979; Menke and Steingass, 1988). Rumen fistulated male buffaloes maintained on 2 kg conventional concentrate mixture (maize 20, wheat 15, deoiled mustard cake 10, mustard cake 10, soybean meal 15, rice bran 15, deoiled rice bran 12, mineral mixture 2, common salt 1part), 5 kg green and *ad lib* wheat straw were used as a donor for rumen liquor. Two sets of samples were incubated in triplicates. In the Ist set, about 375 mg of the ground sample (dry matter basis) was incubated at 39°C for 24h in triplicate in 100 ml calibrated glass syringes with buffered rumen fluid for assessing the net gas production, digestibility of nutrients and metabolizable energy (ME) availability. Individual volatile fatty acids were determined by using GLC equipped with a glass column (6 ft length and 1/8 inch diameter) packed with chromosorb 101. Samples were prepared by adding 0.2 ml of 25% metaphosphoric acid per ml of rumen liquor, allowing it to stand for 2 h followed by centrifugation at 4000 rpm for 7 min. Supernatant was used for estimation of individual volatile fatty acids (IVFA).

In the 2nd set, total gas production was recorded after 24 h of incubation. From the headspace of each syringe, 100 µl gas was collected by puncturing the silicon tube and injected in gas chromatograph for the estimation of methane. Standard calibration gas (Sigma gases, New Delhi) consisted of equal proportion of methane and carbon dioxide.

The flow rates for nitrogen, hydrogen and zero air were 30, 30, 320 ml/min respectively. Blank and standard hay (berseem hay) were run in triplicate with each set.

Statistical analysis

The data were subjected to one-way analysis of variance procedure using SPSS (2012), using the linear model. The post-hoc comparison of means was done for the significant difference by Tukey's b. Significant differences of treatments were considered at P<0.05 level.

Results and Discussion

Chemical composition of concentrate mixtures with graded levels of RDDGS, % DM basis

The CP of concentrate mixture 1 (control)and concentrate mixtures with graded levels of RDDGS varied from 21.53 % to 22.21 % (Table 2). All the concentrate mixtures formulated were iso-nitrogenous.

The ether extract content of concentrate mixtures varied between 4.73% and 5.08% and increased slightly with increase in the inclusion of RDDGS replacing SBM. The NDF content in concentrate mixtures varied from 30.13% to 35.33%.The total carbohydrates (TCHO) in concentrate mixture 1 (control) was 65% while in RDDGS containing concentrate mixtures, it varied from 64.85 to 65.61%. The ADICP and NDICP in concentrates increased with increase in the level of RDDGS in the ration.

In vitro evaluation of concentrate mixture containing graded levels of RRDGS

Net gas production

The inclusion of graded levels of RDDGS in the concentrate mixtures didn't show any significant effect on the net gas production (Table 3). The NGP ranged from 207.83 to 222.08 ml/g DM/24 h. The results in the present study are in agreement with the studies of Segers *et al.,* (2014) which revealed no effect of DDGS supplementation on NGP. However, present results of NGP were not in agreement with Yogi *et al.,* (2017) where the NGP decreased (P<0.01) with incremental levels of RDDGS replacing oil seed cakes as well as with Pecka Kielb *et al.,* (2015) where the total gas production increased (P<0.01) with inclusion of corn DDGS in diet.

Truly degraded substrate (TDS) (mg) of the concentrate mixtures didn't differ significantly with inclusion of graded levels of RDDGS in the concentrate mixtures (Table 3). The TDS (mg) of concentrate mixtures ranged between 346.82 and 347.21.The inclusion of graded levels of RDDGS in concentrate mixtures didn't show any significant effect on partitioning factor (PF) in the concentrate mixtures. The PF of concentrate mixtures ranged from 3.61 to 3.84.The PF is the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (ml) produced. A higher partitioning factor means that proportionally more of the degraded matter is incorporated into microbial mass i.e. the efficiency of microbial protein synthesis is higher. The partitioning factor calculated *in vitro* provides useful information for predicting the dry matter intake, microbial mass production in the rumen and the methane emission of the ruminant animal. The PF of ruminant diets should be in the range of 2.71-4.41 (Blummel *et al.,* 1997). The PF in the present study ranged between 3.61 and 3.84 which is within the suggested range.

Nutrient digestibility

The OM digestibility was higher (P<0.05) in concentrate mixture 3 (50% RDDGS) (87.61%) than concentrate mixture 1 (control) and concentrate mixture 2 (25% RDDGS) and it was statistically similar to that in concentrate mixture 4 (75% RDDGS) (86.47%) and concentrate mixture 5 (100% RDDGS) (86.46%) (Table 3). The neutral detergent fiber digestibility (NDFD %) was lower (P<0.05) in concentrate mixture 1 (control)(48.20%)and concentrate mixture 2 (25% RDDGS) (48.42%) as compared to other concentrate mixtures. The digestibility of DM, NDF and OM of the concentrates containing 50%, 75% and 100% RDDGS levels replacing SBM was higher (P<0.05) than concentrate mixture 1 (control) in the present study. However, Geron *et al.,* (2017) reported no effect of inclusion of DDGS on IVDMD and IVOMD. Contrarily, Yogi *et al* (2017) showed that IVDMD decreased (P<0.01) after 50% inclusion of RDDGS replacing oil cakes, whereas IVOMD was unaffected except at 100% RDDGS where it was reduced.

The microbial mass production (MMP, mg) of concentrate mixtures ranged between 112.37 and 127.95 (Table 3). The MMP of concentrate mixtures with graded levels of RDDGS increased (P>0.05) non significantly with increasing concentrations of RDDGS replacing SBM. However, Yogi *et al* (2017) reported a significant increase (P<0.01) in the MBP with the inclusion of RDDGS replacing oil seed cakes in the diets. The inclusion of graded levels of RDDGS in concentrate mixtures didn’t show any significant effect on efficiency of microbial mass production (EMMP) in the concentrate mixtures. The EMMP of concentrate mixtures ranged from 39.04 to 42.63% which increased with increasing levels of RDDGS in concentrate mixtures replacing SBM numerically, however, the increase was statistically non-significant.

The inclusion of graded levels of RDDGS in concentrate mixtures didn’t show any significant effect on short chain fatty acid production (SCFA) in the concentrate mixtures (Table 3). The SCFA (mmole) ranged between 0.92 and 0.93 among the concentrate mixtures evaluated. The availability of metabolisable energy (ME, MJ/kg DM) was lower (P<0.05) in concentrate mixture 2 (25% RDDGS) (9.79) and concentrate mixture 1 (control) (9.82) (Table 3). The ME was higher (P<0.05) in concentrate mixture 3 (50% RDDGS) (10.33) as compared to concentrate mixture 1 (control). Beyond 75% RDDGS inclusion replacing SBM, the ME decreased (P<0.05) in concentrate mixture 5 (100% RDDGS) (9.80), however, it was statistically similar to that in concentrate mixture 1 (control) and concentrate mixture 2 (25% RDDGS).The ME availability was highest (P<0.05) in concentrate 3 and 4 containing 50 and 75 % RDDGS, respectively replacing SBM.

The ammonical-N of the concentrate mixtures decreased (P<0.05) with the increase in the inclusion of graded levels of RDDGS in concentrate mixtures replacing SBM (Table 3). The ammonical-N (mg/dl) was highest (P<0.05) in concentrate mixture 1 (control) (30.38 mg/dl) and in concentrate mixture 2 (25% RDDGS) (28.78 mg/dl) as compared to other concentrate mixtures. The ammonical-N in concentrate mixture 3 (50% RDDGS) (25.67 mg/dl) was higher (P<0.05) than concentrate mixture 4 (75% RDDGS) (23.56 mg/dl) followed by that in concentrate mixture 5 (100% RDDGS) (22.16mg/dl). Ruminal ammonia-N (NH_3-N), which is the main source of N for microbial protein synthesis, results from microbial degradation of rumen degradable protein (RDP) (Reed *et al.,* 2006). The results are in accordance with the results obtained by Walter *et al.,* (2012) and Yogi *et al.,*(2017) where the NH_3-N decreased (P<0.01) with inclusion of DDGS in the diets. This might be due to the higher rumen undegradable protein in RDDGS which resulted in lower proteolytic activity in rumen leading to lower ammonia production.

Individual VFA production

The TVFA production decreased (P<0.05) with increasing level of RDDGS in the concentrate mixtures (Table 4). The TVFA (mM/dl) production was highest (P<0.05) in concentrate mixture 1 (control) (6.73) as compared to the concentrate mixtures containing graded levels of RDDGS. The TVFA production in concentrate mixture 2 (25% RDDGS) (6.04) was higher (P<0.05) as compared to the concentrate mixtures containing 50%, 75% and 100% RDDGS replacing SBM. The results in the present study are in agreement with results observed by Walter *et al.*,(2012) where the TVFA concentration decreased (P=0.03) linearly with the inclusion of wheat DDGS. The results obtained are also in agreement with Mista *et al.*, (2014) and with Yogi *et al.*, (2017) where the TVFA concentration decreased (P<0.05) with incremental levels of corn DDGS and RDDGS, respectively in the rations.

The acetic acid content (mM/dl) of the concentrate mixtures followed the same trend as TVFA and decreased (P<0.05) with increasing level of RDDGS replacing SBM in the concentrate mixtures (Table 4). The acetic acid (mM/dl) content was highest (P<0.05) in concentrate mixture 1 (control) (3.96 mM/dl) followed by concentrate mixture 2 (25% RDDGS) (3.50), concentrate mixture 3 (50% RDDGS) (3.28), concentrate mixture 4 (75% DDGS) (3.09) and lowest (P<0.05) in concentrate mixture 5 (100% RDDGS) (2.98). In the present study, the acetic acid concentration reduced with inclusion of RDDGS. Our results are in accordance with the results obtained by Yogi *et al.*, (2017) whereas the results obtained by Walter *et al* (2012) and Mista *et al.*, (2014) have showed no significant effect on acetic acid concentration where the grains were replaced by wheat DDGS and corn DDGS, respectively.

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

Ingredient	CONC 1 (Control)	CONC 2 (25% RDDGS)	CONC 3 (50%RDDGS)	CONC 4 (75% DDGS)	CONC 5 (100% RDDGS)
Maize	34	34	34	34	34
SBM	15	11.25	7.5	3.75	0
DDGS	0	3.75	7.5	11.25	15
Mustard Cake	15	15	15	15	15
Wheat Bran	10	10	10	10	10
Rice Polish	6	6	6	6	6
DORP	17	17	17	17	17
Mineral Mixture	2	2	2	2	2
Common Salt	1	1	1	1	1

Table.2 Chemical composition of concentrate mixtures with graded levels of RDDGS, % DM basis

Parameters	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5
OM	91.75	91.88	92.32	92.22	92.18
CP	22.01	22.19	22.21	22.03	21.53
EE	4.73	4.84	5.08	5.01	5.04
Total ash	8.25	8.12	7.68	7.78	7.82
NDF	30.13	31.33	31.67	33.73	35.33
ADF	13.27	12.17	13.53	12.77	14.67
Hemicellulose	16.87	19.17	18.13	20.97	20.67
ADL	4.23	4.40	4.30	4.60	4.77
TCHO	65.00	64.85	65.03	65.17	65.61
ADICP	4.96	5.84	7.18	8.88	8.50
NDICP	9.18	11.91	14.55	16.08	16.13

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 in concentrate mixtures containing graded levels of RDDGS (24h)

Parameters	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
NGP, ml/g/ 24h	210.98	207.95	222.08	217.13	207.83	2.07
TDS, mg	346.82	346.88	347.12	347.21	347.10	0.30
PF, mg/ml	3.61	3.64	3.64	3.67	3.84	0.13
OMD, %	82.99^{a}	83.42^{a}	87.61^{b}	86.47^{b}	86.46^{b}	0.71
NDFD, %	48.20^{a}	48.42^{a}	63.88^{b}	63.00^{b}	64.67^{b}	2.58
MMP, mg	112.37	113.19	120.43	120.36	127.95	2.19
EMMP, %	39.04	39.59	39.60	40.09	42.63	0.53
DMD, %	84.39^{a}	83.84^{a}	88.56^{b}	87.5^{b}	87.52^{b}	0.65
SCFA, mmole	0.93	0.92	0.98	0.96	0.92	0.01
ME, MJ/ kg DM	9.82^{a}	9.79^{a}	10.33^{b}	10.12^{ab}	9.80^{a}	0.08
NH_3-N, mg/dl	30.38^{c}	28.78^{c}	25.67^{b}	23.56^{a}	22.16^{a}	1.04

NGP- Net gas production, TDS-truly degraded substrate, PF- partition 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, ME- metabolizable energy NH_3-N-ammonical nitrogen, Means bearing different superscripts in a row differ significantly ($P<0.05$)

Table.4 *In vitro* volatile fatty acids production (mM/dl) in concentrate mixtures containing graded levels of RDDGS

Parameters	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
Acetate	3.96^{e}	3.50^{d}	3.28^{c}	3.09^{b}	2.98^{a}	0.12
Propionate	1.97^{c}	1.85^{b}	1.74^{a}	1.74^{a}	1.68^{a}	0.03
Isobutyrate	0.074^{c}	0.068^{bc}	0.068^{bc}	0.060^{a}	0.065^{ab}	0.002
Butyrate	0.31^{b}	0.29^{b}	0.27^{ab}	0.24^{a}	0.28^{ab}	0.01
Isovalerate	0.286^{c}	0.228^{b}	0.213^{ab}	0.238^{b}	0.179^{a}	0.012
Valerate	0.12^{d}	0.11^{c}	0.10^{b}	0.08^{a}	0.08^{a}	0.01
TVFA	6.73^{e}	6.04^{d}	5.67^{c}	5.44^{b}	5.26^{a}	0.17
A:P	2.01^{c}	1.89^{b}	1.89^{b}	1.78^{a}	1.77^{a}	0.03
Relative proportion, %						
Acetate	58.87^{c}	57.89^{b}	57.90^{b}	56.79^{a}	56.57^{a}	0.28
Propionate	29.30^{a}	30.60^{b}	30.70^{b}	31.92^{c}	32.02^{c}	0.34
Isobutyrate	1.10^{a}	1.12^{a}	1.20^{ab}	1.11^{a}	1.23^{b}	0.02
Butyrate	4.66	4.80	4.73	4.39	5.29	0.11
Isovalerate	4.25^{b}	3.77^{ab}	3.76^{ab}	4.37^{b}	3.40^{a}	0.13
Valerate	1.82^{b}	1.81^{b}	1.70^{b}	1.42^{a}	1.49^{a}	0.06

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

Table.5 Methane production from fermentation of concentrate mixtures containing graded levels of RDDGS (24 h)

Parameters	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
CH_4,ml	5.51	6.39	6.78	5.72	5.64	0.19
CH_4, ml/g DM	27.54	31.91	33.88	28.62	28.19	0.95
CH_4,ml/100mg DMD	3.31	3.83	3.85	3.31	3.27	0.11
CH_4,ml/100mg OMD	3.67	4.23	4.22	3.64	3.59	0.12

Table.6 Hydrogen balance of concentrate mixtures containing graded levels of RDDGS (24h)

Parameters	CONC 1	CONC 2	CONC 3	CONC 4	CONC 5	SEM
H-recovery, %	91.33^{a}	97.88^{b}	101.35^{c}	103.36^{c}	107.88^{d}	1.87
H-consumed via CH_4	6.16^{d}	5.68^{c}	5.32^{b}	5.00^{a}	$5.20a^{b}$	0.14
FE, %	77.83^{a}	78.41^{b}	78.44^{b}	79.01^{c}	79.06^{c}	0.15
VFA UI	2.25^{c}	2.14^{b}	2.13^{b}	2.01^{a}	2.05^{ab}	0.03

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

The propionic acid content (mM/dl) was higher (P<0.05) in concentrate mixture 1 (control) (1.97) as compared to the other concentrate mixtures (Table 4). The propionic acid content of concentrate mixture 2 (25% RDDGS) (1.85) was higher (P<0.05) than concentrate mixtures 3, 4 and 5 containing 50%, 75% and 100% RDDGS levels, respectively. The relative proportion of propionic acid was higher (P<0.05) in concentrate mixture 4 (75% RDDGS) and concentrate mixture 5 (100% RDDGS) than concentrate mixture 1 (control), concentrate mixture 2 (25% RDDGS) and concentrate mixture 3 (50% RDDGS).

The A: P ratio of concentrate mixture 1 (control) (2.01) was higher (P<0.05) as compared to other concentrate mixtures (Table 4). The A: P ratio of concentrate mixture 2 (25% DDGS) and concentrate mixture 3 (50% RDDGS) was higher (P<0.05) than concentrate mixture 4 (75% RDDGS) (1.78) and concentrate mixture 5 (100% RDDGS) (1.77). The propionic acid content was lower (P<0.05) in the diets containing different levels of RDDGS whereas the relative proportion of propionate has increased (P<0.05) with the increase in the inclusion level of RDDGS and subsequently the A: P ratio decreased (P<0.05) with the RDDGS inclusion in the ration. The results obtained in the present study are in agreement with the results obtained by Yogi *et al.*, (2017) where oil cakes were replaced by graded levels of RDDGS.

The isobutyrate content decreased (P<0.05) with increasing level of RDDGS in the concentrate mixtures (Table 4).

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