International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 11, Issue 6, 2019, pp.-8074-8077.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

IN VITRO EVALUATION OF DIETS OF BUFFALOES CONTAINING DRIED DISTILLERS GRAINS WITH SOLUBLES AS A SUBSTITUTE FOR SOYBEAN MEAL

CHANDRIKA R.N., JASMINE KAUR*, LAMBA J.S., HUNDAL J.S. AND GREWAL R.S.
Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, 141004, India
*Corresponding Author: Email - sachdeva_jasmine@rediffmail.com

Received: March 08, 2019; Revised: March 24, 2019; Accepted: March 25, 2019; Published: March 30, 2019

Abstract: *In vitro* studies were carried out to investigate the effect of using dried distillers grains with solubles (cereal milling by-product) as a substitute for soybean meal (SBM) in the diet of buffaloes. SBM comprising 6% of the whole diet (total mixed ration; TMR) was substituted by rice dried distillers' grains with solubles (RDDGS) at five levels (0, 25, 50, 75 and 100%) on N basis. In vitro dry matter disappearance (IVDMD), organic matter disappearance (IVOMD), net gas production (NGP), methane production and fermentation efficiency of TMRs was studied. RDDGS substitution at 25, 50 and 75% significantly increased (P<0.05) IVOMD and IVDMD. The NH_3-N decreased (P<0.05) with the increasing level of RDDGS in the TMRs. The relative proportion (%) of acetate declined (P<0.05) and that of propionate increased (P<0.05) with the increasing level of RDDGS in the TMRs and subsequently the A: P ratio declined (P<0.05) with RDDGS inclusion beyond 25% level replacing SBM in the TMR. No significant effect of RDDGS inclusion was observed on methane production. The fermentation efficiency increased (P<0.05) with the increasing level of RDDGS in TMRs. In conclusion, RDDGS could replace up to 75% of the soybean meal in the TMR of buffaloes which is equivalent to 4.5% of the whole dietary DM.

Keywords: *Dried distillers grains with solubles, In vitro digestibility, TMR*

Citation: Chandrika R.N., *et al*., (2019) *In vitro* Evaluation of Diets of Buffaloes Containing Dried Distillers Grains with Solubles As A Substitute for Soybean Meal. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 11, Issue 6, pp.- 8074-8077.

Academic Editor / Reviewer: Manjula Thakur

Introduction

Livestock and dairy plays an important role in the economy and livelihood of people in India. Livestock contributes 25.6% of total value of output in agriculture which is 4.11% of total GDP [1]. 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 is increasing. Thus, there is a need to explore various alternate feed resources to meet the standards of feeding. Rice dried distillers grains solubles (RDDGS) is the major co-product from alcohol and rice wine production using broken rice in Asian countries. Scanty information is available on the use RDDGS in the diet of ruminants. Thus, the present study was planned to evaluate the *In vitro* nutritional worth of total mixed rations of buffaloes containing graded levels of rice DDGS.

Materials and methods

Sample collection and preparation

Soybean meal comprising 6 % of the total mixed ration (TMR) was replaced by RDDGS at 0, 25, 50, 75 and 100% levels on N basis [Table-1]. Roughage: concentrate ratio in TMRs was 60:40 ratio on DM basis. All the TMRs contained oats fodder and wheat straw in 1:1 ratio.

Chemical analysis

TMRs were analyzed for dry matter (DM), Kjeldahl N, ether extract (EE) and ash content using the standard procedures [2]. Cell wall fractions, *viz*. NDF, ADF, cellulose and lignin were estimated sequentially using the standard procedure [3]. NDF and ADF were expressed inclusive of residual ash. Lignin was determined by solublization of cellulose with72 percent sulphuric acid. Acid detergent insoluble protein and neutral detergent insoluble protein were determined [4].

In vitro gas production study

The nutritional worth of various TMRs was assessed by *In vitro* gas production technique [5,6]. 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. 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).

Statistical analysis

The data were subjected to one-way analysis of variance procedure 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

Proximate composition and cell wall fractions of TMRs

The organic matter content in TMRs varied from 91.73 to 91.93% [Table-2]. The crude protein in TMRs varied from 12.40% to 12.64% with different levels of RDDGS supplementation. All the rations were iso-nitrogeneous. The ether extract content of TMRs was between 3.0 to 3.63%. The ash content in all TMRs was between 8.07% to 8.27%. The NDF content varied from 58.33 to 60.93% while ADF values varied from 33.80% to 39.6% in the TMRs evaluated. The total carbohydrates varied from 64.85 to 65.61%. The NDICP and ADICP increased with increase in the level of RDDGS in TMRs

Table-1 *Ingredient composition of TMRs containing graded levels of RDDGS (parts/100 parts), TMR1*

	TMR1 (Control)	TMR 2 (25% RDDGS)	TMR 3 (50% RDDGS)	TMR 4 (75% RDDGS)	TMR 5 (100% RDDGS)
Oats fodder	30	30	30	30	30
Wheat straw	30	30	30	30	30
Maize	13.6	13.6	13.6	13.6	13.6
SBM	6	4.5	3	1.5	0
DDGS	0	1.5	3	4.5	6
Mustard Cake	6	6	6	6	6
Wheat Bran	4	4	4	4	4
Rice Polish	2.4	2.4	2.4	2.4	2.4
DORP	6.8	6.8	6.8	6.8	6.8
Mineral mixture	0.8	0.8	0.8	0.8	0.8
Common Salt	0.4	0.4	0.4	0.4	0.4

Table-2 *Chemical composition of TMRs with graded levels of RDDGS (% DM basis)*

Parameters	TMR 1	TMR 2	TMR 3	TMR 4	TMR 5
OM	91.73	91.73	91.93	91.82	91.75
CP	12.41	12.54	12.64	12.59	12.40
EE	3.00	3.20	3.43	3.40	3.63
Total ash	8.27	8.27	8.07	8.18	8.25
NDF	58.33	58.87	59.47	59.40	60.93
ADF	33.80	34.57	36.17	38.20	39.60
Hemicellulose	24.53	24.3	23.3	21.2	21.33
ADL	5.70	5.60	6.13	6.83	7.13
TCHO	76.35	75.99	75.86	75.83	75.72
ADICP	3.53	3.73	3.88	5.51	5.99
NDICP	5.67	6.54	7.38	8.41	8.73

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 total mixed rations (TMRs) containing graded levels of RDDGS (24 h)*

Parameters	TMR 1	TMR 2	TMR 3	TMR 4	TMR 5	SEM
NGP, ml/g/ 24 h	180.56^{a}	186.67^{ab}	190.00^{b}	185.01^{ab}	183.51^{ab}	1.14
TDS, mg	346.78	344.03	344.78	346.16	344.98	0.38
PF, mg/ml	3.63	3.75	3.62	3.72	3.56	0.03
OMD, %	71.45^{a}	76.31^{b}	74.77^{b}	74.87^{b}	71.16^{a}	0.69
NDFD, %	55.10^{a}	63.08^{b}	60.99^{b}	61.15^{b}	56.57^{a}	1.03
MMP, mg	97.63^{ab}	108.53^{b}	101.03^{ab}	105.72^{b}	93.68^{a}	1.92
EMMP, %	39.39	41.34	39.19	40.79	38.16	0.45
DMD, %	73.81^{a}	78.27^{b}	76.80^{b}	76.92^{b}	73.54^{a}	0.63
SCFA, mmole	0.80^{a}	0.82^{ab}	0.84^{b}	0.82^{ab}	0.81^{ab}	0.01
ME, MJ/ kg DM	7.72^{a}	7.97^{b}	8.15^{b}	7.97^{b}	7.96^{b}	0.05
NH_3-N, mg/dl	20.15^{e}	17.19^{d}	15.39^{c}	15.09^{b}	14.79^{a}	0.67

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, ME- Metabolizable energy, NH3-N-Ammonical nitrogen, roughage : concentrate ratio in TMRs was 60:40 on DM basis, all the TMRs contain oats fodder and wheat straw in the ratio of 1:1. Means bearing different superscripts in a row differ significantly (P<0.05)

Table-4 *In vitro volatile fatty acids production (mM/dl) from TMRs containing graded levels of RDDGS*

Parameters	TMR1	TMR 2	TMR 3	TMR 4	TMR 5	SEM
Acetate	3.16^{c}	2.77^{c}	2.39^{a}	2.43^{a}	2.33^{a}	0.10
Propionate	1.50^{b}	1.36^{a}	1.27^{a}	1.35^{a}	1.31^{a}	0.03
Isobutyrate	0.00	0.00	0.00	0.00	0.00	0.00
Butyrate	0.21^{c}	0.19^{bc}	0.18^{b}	0.15^{a}	0.14^{a}	0.01
Isovalerate	0.244	0.256	0.258	0.250	0.250	0.002
Valerate	0.00	0.00	0.00	0.00	0.00	0.00
TVFA	5.11^{c}	4.58^{b}	4.11^{a}	4.18^{a}	4.03^{a}	0.14
A:P	2.11^{b}	2.03^{b}	1.88^{a}	1.81^{a}	1.78^{a}	0.04
Relative proportion, %						
Acetate	61.86^{c}	60.44^{b}	58.26^{a}	58.18^{a}	57.85^{a}	0.53
Propionate	29.30^{a}	29.75^{a}	30.96^{b}	32.17^{c}	32.43^{c}	0.42
Isobutyrate	0.00	0.00	0.00	0.00	0.00	0.00
Butyrate	4.06^{abc}	$4.22b^{c}$	4.48^{c}	3.67^{ab}	3.51^{a}	0.12
Isovalerate	4.77^{a}	5.59^{b}	6.29^{c}	5.98^{bc}	6.20^{c}	0.19
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 superscript in a row differ significantly (P<0.05)

***In vitro* evaluation of TMRs containing graded levels of RRDGS**

Net gas production (NGP): NGP was lowest (P<0.05) in TMR 1 (control) whereas the NGP was highest (190 ml/g DM/ 24hr) in TMR 3 (50% RDDGS replacing SBM). However, the NGP in TMR 2 (25% RDDGS), TMR 4 (75% RDDGS), and TMR 5 (100% RDDGS) was similar. The NGP in the present study was observed to increase with RDDGS inclusion in TMRs being highest (P<0.05) at 50% RDDGS level replacing SBM in TMR 3. The results in the present study are in agreement with the results obtained by [7].

Table-5 *Methane production from fermentation of TMRs containing graded levels of RDDGS (24 h)*

Parameters	TMR1	TMR 2	TMR 3	TMR 4	TMR 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	29.19	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 TMRs containing graded levels of RDDGS, (24 h)*

Parameters	TMR1	TMR 2	TMR 3	TMR 4	TMR 5	SEM
H-recovery, %	105.01^{a}	113.15^{b}	122.68^{c}	118.23^{bc}	120.31^{d}	2.13
H-consumed via CH_4	4.65^{c}	4.19^{b}	3.81^{a}	3.72^{a}	3.53^{a}	0.13
FE, %	77.36^{a}	77.73^{a}	78.46^{b}	78.86^{b}	79.01^{b}	0.22
VFA UI	2.39^{c}	2.32^{c}	2.17^{b}	2.04^{a}	2.00^{a}	0.05

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

Where the total gas produced increased (P<0.01) with inclusion of DDGS in diets. However, present results of NGP are not in agreement with [8] where the NGP decreased with incremental levels of RDDGS. The inclusion of graded levels of RDDGS in TMRs didn't have any significant effect on truly degraded substrate (TDS) and partitioning factor (PF) in the TMRs (Table 3). The TDS (mg) of the rations ranged from 344.03 to 346.78mg. The partitioning factor (PF) of TMRs ranged between 3.56 to 3.75. 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 *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 [9]. The PF in the present study ranged between 3.56 and 3.75 which are well within the suggested range.

Nutrient digestibility

The organic matter digestibility (OMD %) was higher (P<0.05) in TMR 2 (25% RDDGS), TMR 3 (50% RDDGS) and TMR 4 (75% RDDGS) than control TMR 1 [Table-3]. The OMD % was lower (P<0.05) in TMR 5 (100% RDDGS) (71.16%) as compared to other total mixed rations evaluated but statistically it was similar to that in TMR 1 (control). The NDF digestibility (NDFD%) in TMR 2 (25% RDDGS), TMR 3 (50% RDDGS) and TMR 4 (75% RDDGS) was higher (P<0.05) than TMR 1 (control), however, beyond 75% RDDGS level, the NDFD % decreased (P<0.05) in TMR 5 containing 100% RDDGS replacing SBM. The IVDMD and IVOMD of the TMRs with 25 %, 50% and 75% RDDGS levels replacing SBM were higher (P<0.05) than control (TMR 1) in the present study. However, [10] reported no effect of inclusion of DDGS on IVDMD and IVOMD replacing corn and soybean meal [8]. 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) followed a trend similar to that of OM and NDF digestibility and was lower (P<0.05) in TMR 5 (containing RDDGS completely replacing soybean meal) than other TMRs evaluated. However, [8] reported that MMP was highest at 50% and 100% level of inclusion of RDDGS replacing oil seed cakes. In the present study, the efficiency of microbial mass production (EMMP %) in the TMRs ranged from 38.16 to 41.34. The EMMP was numerically higher in TMR 2 (41.34) followed by TMR 4 (40.79), TMR 1 (39.39), TMR 3 (39.19) and lower in TMR 5 (38.16). However, graded levels of RDDGS in the TMRs had no significant effect on the EMMP. The short chain fatty acid production (SCFA) (mmole) was higher (P<0.05) in TMR 3 (0.84) as compared to TMR 1 (control) [Table-3]. The metabolisable energy (ME, MJ/kg DM) was higher (P<0.05) in TMRs containing graded levels of RDDGS compared to TMR 1 (control). The NH_3-N decreased (P<0.05) with the increasing level of RDDGS in the TMRs. The NH_3-N (mg/dl) production was highest (P<0.05) in TMR 1 (20.15), followed by TMR 2 (17.19), TMR 3 (15.39), TMR 4 (15.09) and lowest (P<0.05) in TMR 5 (14.79). Ruminal NH_3-N is the main source of N for microbial protein synthesis and results from microbial degradation of rumen degradable protein [11]. The results of the present study are in accordance with the results obtained by [12] and [8]. This reason for decline in NH_3-N might be 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 (mM/dl) production was higher (P<0.05) in TMR 1 (control) (5.11) as compared to the TMRs containing graded levels of RDDGS [Table-4]. In the current study, the amount of TVFA decreased (P<0.05) with the inclusion of RDDGS in the TMRs upto 50% level of RDDGS replacing SBM. The results are in agreement with those observed by [8] and [12] where the TVFA concentration decreased linearly with inclusion of wheat DDGS. The reason might be the reduced availability of non-structural carbohydrates in RDDGS containing rations. The acetic acid (mM/dl) production was higher (P<0.05) in TMR 1 (control) (3.16) as compared to treatment TMRs [Table-4]. The relative proportion (%) of acetic acid production also followed the same trend as that of acetic acid produced with highest (P<0.05) relative proportion in TMR 1 (61.86) followed by TMR 2 (60.44), TMR 3 (58.26), TMR 4 (58.18) and TMR 5 (57.85), indicating that acetic acid production followed a declining trend with increasing level of RDDGS in the TMR. Thus, in the present study, the acetic acid levels reduced (P<0.05) with inclusion of RDDGS in the TMRs. This may be due to the fact that fermentation of a feed like RDDGS containing small cell wall fraction is likely to produce a lower molar proportion of acetate. Our results are in accordance with the results obtained by [8] whereas the results obtained by [12] and [13] showed no significant effect on acetic acid concentration where the cereal grains were replaced by wheat DDGS and corn DDGS, respectively. The propionic acid (mM/dl) production was higher (P<0.05) in control TMR 1 (1.50) as compared to TMRs containing graded levels of RDDGS. The propionic acid content (mM/dl) was lower (P<0.05) in the TMRs containing graded levels of RDDGS whereas the relative proportion (%) of propionate in the TVFAs 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 beyond 25% level replacing SBM in the ration. The results obtained in the present study are in agreement with [8], where the proportion of propionate increased and that of acetate decreased (P<0.01) with increase in level of RDDGS and there was decrease (P<0.01) in A:P ratio with increasing levels of RDDGS in substrate. The butyrate content decreased (P<0.05) with the increase in the RDDGS inclusion in the diets. This is in relevance with the findings of [7] where increasing levels of DDGS reduced (P<0.01) the butyrate content whereas [12] reported increased butyrate content with both wheat and corn DDGS. However, [13] reported that replacing crushed cereals and oil cakes with DDGS had no significant effect on butyrate content *In vitro*. The isovalerate (mM/dl) production was not affected by graded levels of RDDGS in the TMRs with the concentration ranging between 0.244 and 0.258 [Table-4]. However, [7] and [13] reported decrease (P<0.05) in the isovalerate content with inclusion of DDGS. In the current study, isobutyrate and valerate in the TMRs were present in negligible amounts to be detected.

Methane production

The inclusion of graded levels of RDDGS in the TMRs had no significant effect on the methane production [Table-5]. Contrary to our results, [13] reported a drop in methane production with inclusion of DDGS. However, the results of the present study are in agreement with the results obtained by [7] where there was no significant effect on methane production with inclusion of DDGS in diets.

Hydrogen balance: Hydrogen recovery (%) was observed to be higher (P<0.05) in RDDGS based TMRs as compared to TMR 1 (control) [Table-6]. Hydrogen consumed via CH4 reduced (P<0.05) with the inclusion of RDDGS in the TMRs. The consumption of hydrogen via methane was observed to be lowest (P<0.05) in TMR 3 (50% RDDGS), TMR 4 (75% RDDGS) and TMR 5 (100% RDDGS). Fermentation efficiency (%) was higher (P<0.05) in TMR 3, TMR 4 and TMR 5 containing 50%, 75% and 100% RDDGS, respectively replacing SBM (N basis) as compared to TMR 1 (control) [Table-6]. The fermentation efficiency increased (P<0.05) with the increasing level of RDDGS in TMRs which is similar to the results obtained by [8] who reported an increase (P<0.01) in the fermentation efficiency as the oil seed cakes were replaced by RDDGS *In vitro*. VFA utilization index was lower (P<0.05) in TMRs containing graded levels of RDDGS as compared to TMR 1(control) [Table-6]. VFA utilization index (NGGR) followed reverse trend of fermentation efficiency. The non-glucogenic to glucogenic VFA ratio (NGGR) is associated with effects on methane production, milk composition and energy balance. Glucogenic propionate contributes to energy deposition in body tissues, where non-glucogenic acetate and butyrate are sources for long-chain fatty acid synthesis. Too high VFA UI (NGGR) indicates a high loss of energy in the form of gases [14]. The low value of NGGR indicates the best utilization of VFAs.

Conclusion

The data conclusively revealed that RDDGS inclusion upto 75 percent in the TMRs replacing soybean meal (% N basis) improved (P<0.05) the digestibility of nutrients (OM, NDF and DM), microbial mass production and fermentation efficiency.

Application of research: Study was planned to evaluate the *In vitro* nutritional worth of total mixed rations of buffaloes containing graded levels of rice DDGS

Research Category: Animal Nutrition

Acknowledgement / Funding: Authors are thankful to Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, 141004, India

***Research Guide or Chairperson of research: Dr Jasmine Kaur**
University: Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, 141004
Research project name or number: PhD Thesis

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, 141004

Animal name: Buffalo

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, 141004, India.

Ethical Committee Approval Number: IAEC:532-559 DTD 20.07.2016

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