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Research Article

IN VITRO EVALUATION OF FORMALDEHYDE TREATED RAPESEED MEAL FOR RUMEN FERMENTATION VARIABLES

CHOUBEY M., SORATHIYA K.K.*, PATEL V.R., JADHAV M.D. AND RAVAL A.P.

Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, Navsari, 396 450, India
*Corresponding Author: Email-sorathiya.kk@gmail.com

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Abstract- The present study was planned to evaluate the inclusion of graded level of formaldehyde treated dietary protein (rapeseed meal) for ruminant feeding through in vitro gas production technique. Five different type of concentrate mixtures were prepared by replacing protein ingredient (rapeseed meal) from basal concentrate mixture at graded level (0, 25, 50, 75 and 100%) with that of formaldehyde treated one. The respective diet prepared from these concentrate mixture were incubated with buffalo rumen liquor at ruminal temperature to assess the fermentation variables and nutrient utilization efficiency. The formaldehyde treatment of rapeseed meal resulted into improved production of microbial biomass with an enhanced tendency for its efficiency at the level of 50% and above. It has also prevented the microbial degradation of dietary protein as evident by lowering of ammonia nitrogen at dose level higher than 50%. The replacement of rapeseed meal with that of formaldehyde treated one in concentrate mixture have potential to protect the microbial degradation of protein in rumen with optimistic effect on microbial biomass production and rumen ammonia.

Keywords- Bypass protein, Microbial biomass, Nutrient utilization, Partitioning factor, Rumen metabolites

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Introduction

The main objective of dairy farming system is to get maximum produce from animals in a cost effective manner. Among all production cost inputs of animal husbandry, feeding alone covers more than 60% and hence requires technological interventions for its optimum utilization [1]. In this regard, protein rich feed ingredient is supposed to be the most expensive and valuable component of animal's diet, because of lower area under oil seed production and export of oilseed cakes in a substantial proportion [2]. Hence, improving the utilization efficiency of protein ingredients in ruminant diet is of special concern. A major fraction of dietary protein consumed by animals is being degraded to ammonia in the rumen, which is being utilized by rumen microbes for synthesis of microbial protein. However, excess ammonia produced from ruminal degradation of dietary protein diffuses to circulation, increasing toxic urea in blood and then excreted as urinary nitrogen leading to wastage of valuable nutrient [3]. Thus, to overcome the mentioned shortcomings of ruminal degradation, the concept of protected (bypass) protein has come up with the aim to prevent the excess microbial degradation of dietary protein in rumen [4]. This technology could be helpful in improving the bioavailability of amino acids at lower gastro-intestinal tract along with prevention of ammonical loss of dietary protein [5]. Above all, this will also help to resolve the environmental impact of animal wastes through lower excretion of nitrogenous end product [6]. Among all methods, formaldehyde treatment of protein ingredients is most common and recommended technique for the aforementioned purpose [2,7]. The *in vitro* rumen fermentation method for evaluation of nutritional appeal of feed resources is one of the novel approach, which enables selection of feed or feed constituents for elevated efficiency of microbial biomass synthesis in the rumen. It provides a basis for development of feeding strategies to optimize ruminant production in a very small time [8, 9]. Keeping the aforesaid points in mind, the present study was planned to evaluate the inclusion level of formaldehyde treated protein (FTP) for ruminant feeding through in vitro gas production technique.

Materials and Methods

Preparation of dietary substrate: Five different type of concentrate mixtures were formulated by replacing protein ingredient (rape seed meal) from basal concentrate mixture at graded level (0, 25, 50, 75 and 100%) with that of formaldehyde treated (@ 1% of CP) one and designated as CON, FTP-25, FTP-50, FTP-75, and FTP-100 [Table-1]. The respective concentrate mixtures were prepared and procured from Mehsana District Cooperative Milk Producers' Union Limited (Dudhsagar Dairy), Mehsana, Gujarat. The representative samples of concentrate mixtures, green and dry fodder were dried in oven at 90ºC for overnight and grounded to pass 1 mm sieve. The three dietary ingredients were then mixed in a ratio of 50:25:25 and stored in air tight container for further use as substrate.

Donor animals and their feeding regimen

Rumen liquor (RL) was collected from three donor male Surti buffalo bulls maintained on basal diet for a period of three weeks at Livestock Research Station, NAU, Navsari. The RL was collected in the early morning before feeding of the donor animals by using a stomach tube. The RL collected in a pre-warmed thermo-flask, brought immediately to the laboratory, strained through a muslin cloth, pooled and kept under CO2 bubbling at 39ºC. This RL was used as inoculum and incubating medium was prepared by addition of buffer mixture to it [10].

Test proper

The different substrates prepared were incubated with inoculum and medium for 24 hours as per standard procedure [10]. Briefly, 200 mg air dried milled substrate was incubated in 100 ml glass syringes with greased (petroleum jelly) piston for smooth movement without any leakage. 30 ml buffered rumen fluid was dispensed in each syringe and clamped after careful removal of air bubbles trapped in by gentle shaking and upward movement of the piston. Initial volume was recorded and syringes were placed vertically in a stand with holes to hold the syringe upright in the incubator ventilated by fan assisted forced air circulation at 39ºC. The gas production was recorded at 24h of incubation with intermittent shaking. At the end of the incubation period (24h), gas production was recorded as the upward movement of position from the initial reading and corrected for blank (rumen fluid). For testing of dietary treatment two sets of three syringes for each sample were prepared, and two syringes as standard. First set of syringes were used to estimate the nutrient utilization efficiency (digestibility) and microbial biomass production while second set was used for estimation of rumen metabolites.

Nutrient profile of substrate

The three dietary components (concentrate, green and dry fodder) used as substrate were analyzed for their proximate principle [11] and fiber fractions (neutral detergent fiber, acid detergent fiber and hemicellulose) as per standard procedure [12].

Nutrient utilization

The DM digestibility was estimated by transferring of syringe contents to a spoutless beaker by repeated washing with 100 ml neutral detergent solution [12]. The flask content was refluxed for 1h and filtered through pre-weighed Gooch crucibles to arrive at DM residue. The in vitro truly degradable organic matter in the rumen (TDOMR) was calculated as the amount of substrate OM incubated minus the amount of substrate recovered as residue after neutral detergent solution treatment: TDOMR = [(Initial OM of feed taken for incubation - NDF residue x100) / (Initial OM of feed taken for incubation)]. The partitioning factor (PF) was calculated as the ratio of TDMOR (mg) to gas volume (ml) produced from it during 24h of incubation: PF = TDOMR mg/ml of total gas produced. Metabolizable energy was calculated as ME=2.20+0.136×gas volume+0.057×CP.

Microbial biomass production and its efficiency

Microbial biomass production (MBP) was worked out as per the equation: MBP (mg) = [TDOMR (mg) - (2.2 x net volume of gas produced)]. Efficiency of microbial biomass production (EMP) expressed as MBP (mg) /100 mg TDOMR, where constant 2.2 is the stoichiometric factor. The partitioning factor (PF) was calculated as the ratio of TDMOR (mg) to gas volume (ml) produced from it during 24h of incubation: PF = TDMOR mg/ml of total gas produced.

Rumen metabolites: From the second set of syringes, incubation medium was strained through muslin cloth to obtain stained rumen liquor (SRL). The ammonia nitrogen and total volatile fatty acid was estimated as per the standard procedure as described elsewhere [13].

Statistical analysis: The data generated were analyzed for their statistical significance using Statistical Package for the Social Sciences (SPSS, version 20.0 Chicago, USA). Data were analyzed using one-way ANOVA to distinguish the impact of different dietary treatments. The effects were considered to be significant at P<0.05 and declared as trend/tendency at 0.05<P<0.10.

Table-1 *Ingredient composition of different concentrate mixture*

Ingredients	CON	FTP-25	FTP-50	FTP-75	FT-100
Damaged wheat	4	4	4	4	4
Maize grit	4	4	4	4	4
Cotton seed ext.	3.5	3.5	3.5	3.5	3.5
Rice polish(fine)	9.9	9.9	9.9	9.9	9.9
Deoiled rice bran	47.1	47.1	47.1	47.1	47.1
Rapeseed meal	10	7.5	5	2.5	--
Rapeseed meal (formaldehyde treated)	--	2.5	5	7.5	10
Rice flake bran	5	5	5	5	5
Molasses	11	11	11	11	11
Sugar booster	0.5	0.5	0.5	0.5	0.5
Urea	1	1	1	1	1
Calcite powder	2	2	2	2	2
Common salt	2	2	2	2	2
Total	100	100	100	100	100

Result and Discussion

The data pertaining to the chemical composition of experimental feed used as substrate for study are presented in [Table-2]. The concentrate mixture contained 18.72 % of crude protein on dry matter basis and inclusion of formaldehyde treated rapeseed did not showed any differences in their proximate composition particularly protein. As usual the fibre fraction i.e. NDF and ADF were higher in the fodder samples as compared to concentrate mixture. The chemical composition of different feed and fodder were in accordance with the earlier reports for Indian feed and fodder [14].

Table-2 *Chemical composition of experimental feed*

Feed	%, dry matter						
	Organic matter	Crude protein	Ether extract	TCHO	NDF	ADF	Hemi-cellulose
CON	88.08	18.72	2.24	67.12	30.25	16.46	13.79
FTP-25	87.62	18.71	2.25	66.66	29.55	17.54	12.00
FTP-50	87.95	18.84	2.23	66.89	28.76	15.75	13.01
FTP-75	87.55	18.68	2.25	66.62	28.92	16.24	12.68
FT-100	87.85	18.85	2.20	66.80	29.05	16.45	12.59
Green fodder	91.04	8.08	2.93	80.04	65.81	36.63	29.18
Dry fodder	89.49	8.04	1.60	79.85	75.07	46.68	28.39

[Table-3] depicts the values regarding the in vitro fermentation characteristics and nutrient utilization. Although in vitro gas production during a period of 24 hour in different dietary treatments did not revealed any differences (P>0.05), however, a reduction in values were noticeable due to increase in level of FTP. Similar to the gas production, there was no significant alteration in the utilization of nutrient variables for dry matter and organic matter. A close perusal of data related to nutrient utilization indicates a numerical drop (P>0.05) in values, which could be well sensed with increasing the level of treatment. As only one ingredient of diet i.e. rapeseed was treated with formaldehyde with aim to protect it from microbial degradation which might have prevented the significant depression in gas production and nutrient utilization variables. Moreover, such protective effects have been found to be associated with the level of formaldehyde treatment for protein ingredients [15].

Microbial biomass production (MBP), which is a combined function of gas production and organic matter digested [16], indicated its optimum value in FTP-50 and FTP-75 group, depicting no further benefit of formaldehyde treatment of protein ingredient above 50% level. Nevertheless, the efficiency of microbial biomass production displayed a progressive trend (0.05<P<0.10) due to

formaldehyde treatment of protein ingredient in concentrate mixture. Likewise MBP, the values for its efficiency again indicated 50% as optimum level of treatment. An optimal balance of rumen degradable and undegradable protein has been recommended under several studies [17, 18]. As rumen microbes require ammonia and amino acids in a fixed proportion along with soluble carbohydrate for their biomass production, thus protection of protein degradability through formaldehyde seems to be beneficial only up to a certain level, as shown in the present experiment. Thus, no additional benefit in terms of microbial biomass could be harvested by inclusion of formaldehyde treated rapeseed meal at higher level in the concentrate mixture as substrate for in vitro gas production test. Partitioning factor (PF) signifies the extent of degraded matter which is being incorporated into microbial mass [8]. A very weak trend (P=0.165) for improvement of PF was observed due to formaldehyde treatment of rapeseed in concentrate mixture.

Table-3 *Effect of formaldehyde treatment of rapeseed meal on in vitro rumen fermentation variables*

Attributes	FT-0	FT-25	FT50	FT-75	FT-100	SEM	P value
NGP, ml	31.83	31.56	26.25	26.05	24.5	2.12	0.214
Nutrient utilization							
IVDMD, %	58.25	56.35	55.38	56.34	54.34	1.95	0.658
TDOMR	132.70	131.37	127.06	128.26	121.68	3.25.	0.265
TDOMR, %	66.35	65.69	63.53	64.13	60.84	2.16	0.289
Microbial biomass production							
Production, mg	62.68a	61.94a	69.31b	71.06b	67.78ab	2.88	0.031
Efficiency, %	47.23	47.15	54.55	55.40	55.70	2.01	0.091
Partitioning factor	4.17	4.16	4.84	4.93	4.97	0.287	0.165
Rumen metabolite							
Ammonia-N, mg/dL	9.42b	9.13b	8.57ab	7.56a	7.64a	1.05	0.042
TVFA, mM/dL	2.53	2.56	2.25	2.16	2.20	0.18	0.345

Among rumen metabolites, there was no variation (P>0.05) in the level of volatile fatty acid production among different dietary treatments. A depression (P<0.05) in ruminal level of ammonia nitrogen from 75% level of inclusion of formaldehyde treated rapeseed meal in concentrate mixture further strengthens and confirms the protein protecting potential of formaldehyde from rumen microbial degradation. Similar findings have been also reported in previously experimental studies [19, 20]. Rumen ammonia level started showing depression in their values above inclusion level of 50% indicating the effective level of inclusion in diet. Terminal amino groups of proteins are supposed to react with formaldehyde through a condensation reaction to form a stable methylene linkage between protein chains, which protects the degradation of dietary protein from rumen microbial enzymes leading to low level of ammonia [21].

Conclusion

From the finding of present experiment it may be concluded that replacement of untreated rapeseed meal with that of formaldehyde treated in concentrate mixture have potential to protect its microbial degradation in rumen with optimistic effect at the level of 50% on microbial biomass production and rumen ammonia. Further in vivo studies may be undertaken for its systematic evaluation under different physiological condition of ruminant animals.

Abbreviations

NGP: Net gas production; IVDMD: *In vitro* dry matter digestibility; TDOMR: Truly degradable organic matter in rumen; TVFA: Total volatile fatty acid

Author Contributions

CHOUBEY M. and SORATHIYA K.K. planned and were involves throughout the experiment including writing of manuscript. PATEL V.R. and JADHAV M.D. helped in analytical process of feed, fodder and rumen liquor. RAVAL A.P. helped in feeding of donor animal and collection of rumen liquor.

Conflict of Interest: Authors declare that they have no Conflict of Interest.

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