



Nature of Carbohydrates in Agro-industrial By-products (AIBP) Scarcely Support Rumen Microbial Protein Production but Sustain Milk Composition

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10.18805/ajdfr.DR-1769

ABSTRACT

Background: The microbial protein (MBP) production in rumen has quantitative influence on protein availability to host animal and also influence milk composition, particularly milk protein. Few native breeds produce low levels of milk that should sustain economically on agro-industrial by-products (AIBP). However, the nature of carbohydrates in AIBP how far support the MBP in rumen is seldom quantified. The objective of the study was to evaluate the cell wall carbohydrates of AIBP as a source of energy for rumen MBP production and milk composition in *Bos indicus* cows.

Methods: Twenty Deoni breed cows in mid-lactation were divided in complete randomized block design into 5 groups of 4 each based on milk yield, body weight, parity and days in lactation. Basal roughage was finger millet straw *ad-lib*. The cows in treatment groups were supplemented with wheat bran (T1), or hyacinth bean grit (T2), or soy husk (T3), or 50% green gram grit plus 50% pigeon pea husk (T4) and compared with a control group (CG) supplemented with sorghum grain. All the supplements were made 18% isonitrogenous with commercial urea and fortified with 2% common salt and 3% minerals and vitamins mix. The diets were fed for 7 weeks including a digestibility trial of 5 days duration.

Result: Total diet intake was comparable between CG and TGs. CP, hemicelluloses, celluloses ($P \leq 0.01$) and, EE ($P \leq 0.05$) digestibility were higher in TGs than CG. Energy efficiency was comparable between grain and AIBPs. Rumen MBP was lesser in TGs than CG. Higher rumen MBP among TGs was observed in T4 (180 g d^{-1}) but it was 45 g d^{-1} lesser than CG. Milk total solids (TS), fat and solids-non-fat (SNF) were comparable between CG and TGs. The coefficient of variation in daily milk yield was reduced in TGs. The concentrate supplement with 50% grit and 50% husk combination was only suitable to moderately sustain rumen MBP production and also support milk production otherwise, AIBP as the sole concentrate supplement had limited benefit for rumen MBP production.

Key words: Brans, Grains, Husk, Milling, Native cattle, Protein, Ration.

INTRODUCTION

The native breeds of cattle are small in size and have substantially low milk yielders. The majority of the population can be seen in Africa and Asia. Indigenous cows are inclined to more cell wall diets than quality diets because of poor digestive plasticity. Their intake of quality proteins had been reported to restrict their quantitative intake (Singh and Srinivas, 2020). The concentrate supplements with above 18% CP mixtures are discouraged in general to the dairy cows vowing to caveats associated with fertility, environmental contamination of excess ammonia in urine, acid load on kidneys, metabolic diseases and some fertility parameters due to increased blood ammonia (Solo, 2018). The fertility of native breeds is important owing to their decreasing population. Because of the genetic, metabolic and physical limitations associated with native breeds, they required to sustain on economical feeds but able to cater the quantitative production of rumen microbial protein (MBP).

Many Agro-industrial by-products (AIBP) contain CP from 14% to 18% and are available during grains/cereals milling like brans, grit, husk, etc., (Rathod and Srinivas, 2018). Concentrate mixtures with CP from 14% to 18% are recommended for the health of dairy cattle (Hynes *et al.*, 2016). However, it is not known that the nature of

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How to cite this article: Rathod, A.J. and Srinivas, B. (2022). Nature of Carbohydrates in Agro-industrial By-products (AIBP) Scarcely Support Rumen Microbial Protein Production but Sustain Milk Composition. Asian Journal of Dairy and Food Research. DOI: 10.18805/ajdfr.DR-1769.

Submitted: 19-06-2021 **Accepted:** 02-03-2022 **Online:** 12-04-2022

carbohydrates present in AIBP how far can cater to the energy needs of rumen microbes and rumen MBP production? Many studies exist on the impact of AIBP on milk and its composition but to the best of our literature search, no studies we could find what could be the impact of AIBP on the rumen MBP production? Apart from the smaller size native breeds where MBP production is proportionate to the size (Srinivas *et al.*, 2018), the inadequacy of soluble and starchy carbohydrates in AIBP may hypothetically impend rumen MBP production. The study was conducted to evaluate the potential impact of sole AIBP based concentrate mixture supplementation on the

rumen MBP in comparison to grain as control besides limited capacity of milk yield in native Deoni (*Bos indicus*) breed of cows.

MATERIALS AND METHODS

Experiments were conducted at the Livestock Research Centre, Southern Regional Station, ICAR-National Dairy Research Institute (NDRI), Bangalore, India in the year 2014. The latitude, longitude and elevation of the experimental place are 12.947014°N (12° 56' 49.2504" N), 77.607679 (77° 36' 27.6444" E) and 921 m MSL, respectively. The tropical climate is considered to be Aw (Savanna, wet) according to the Köppen-Geiger climate classification with a mean temperature of 23.6°C and 831 mm annual rainfall. The variation in temperatures throughout the year is 6.4°C. During the experimental period, the relative humidity ranged from 59 to 73%, the mean ambient temperature was 72 to 79°F and the mean heat index was 77 to 82°F. The experiment was carried with the approval of the Institute Animal Ethics Committee (IAEC) and reared as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments (CPCSEA), New Delhi, India.

Twenty Deoni cows in 116±9 d of lactation were distributed in a completely randomized block design to 5 groups with 4 replicates based on mean milk yield (3.63±0.26 kg), body weight (352±9 kg) and parity (3.5±0.5). Cows housed in well ventilated, individual pens and provided *ad lib* water 6 times a day and dewormed before starting the experiment. The basal roughage was finger millet (*Eleusine coracana*) straw (FMS) and fed *ad-lib* twice a day. The balanced nutrients (NRC, 2001) were met through a concentrate supplement consisted 92.7% sorghum grain as control (CG) or treatment groups (TG) consisted of 93.24% wheat bran (T1), 94.10% hyacinth bean grit (T2), 92.74% soy husk (T3), or 91.94% green gram grit and Bengal gram husk in 50:50 ratio (T4). All 5 types of concentrate supplements were made iso-protein (18%) by balancing with commercial urea at the rate of 2.93%, 1.76%, 2.26%, 0.90% and 3.05% in CG and TGs, respectively. All the concentrate feed mixtures (CFM) were fortified with 2% common salt and 3% minerals and vitamins mix (M/s Neospark Drugs and Chemicals Pvt. Ltd., India). Concentrate mixture was offered 2.4 kg/day/cow in 2 equal parts soon after milking at 6 AM and 5 PM every day.

After 4 weeks of the preliminary period of feeding, a digestibility trial for 5 days was conducted. Total faeces collected in the 24 hours cycle were weighed, a part was kept for oven drying at 100±1°C overnight and another part was acidified with 25% H₂SO₄ (v/v) in a glass bottle for nitrogen estimation. Spot urine from the cows was collected 100 mL/day in a bucket acidified with 10% H₂SO₄ (v/v). Acidified urine samples were diluted with distilled water (DW) uniformly to 1.2 L, filtered through glass wool and 50 mL was stored at -20°C in polypropylene bottles. FMS, CFM, orts and faeces samples were processed through a cutting

Willey mill using a 1 mm sieve. These samples were analyzed for proximate chemical and cell wall constituents according to AOAC (2012). Total carbohydrates (TCHO), non-fibrous carbohydrates (NFC) and hemicelluloses were mathematically calculated (VanSoest *et al.*, 1991). Energy parameters were calculated using empirical formulae (ARC, 1980). Milk yield was recorded daily and expressed as 4% fat corrected milk (FCM). Morning and evening milk samples were analyzed for total solids (TS), milk fat, milk protein and solids-non fat (SNF) as per the standard methods (AOAC, 2012).

Rumen MBP production was quantified based on the urinary excretion of creatinine and purine derivatives (Chen *et al.*, 1995). Diluted spot urine samples were thawed and treated with uricase to degrade uric acid to allantoin and other compounds. Uricase (U-9375, M/s Sigma Chemicals Co, USA) was prepared in phosphate buffer (0.67 M KH₂PO₄, adjusted the pH with KOH) to obtain a concentration of 0.12 units/mL. One ml of phosphate buffer was added to 2.5 mL of diluted urine sample in the test tube and mixed thoroughly to add 150 µL of uricase solution with pH adjusted to 9.0 by NaOH. Tubes were incubated for 2 hours at 37°C. Total allantoin and creatinine in urine samples were determined by the colorimetric method (Chen *et al.*, 1995). The duodenal flow of MBP was calculated based on the total purine derivatives (PD) and creatinine (PDC) index. The endogenous contribution of PD in indigenous cattle was taken as 0.147 W^{0.75} per day (Chen and Ørskov, 2005). The efficiency of MBP production was expressed as gN/kg of digestible OM intake (DOMI) and gN/MJ ME.

Weekly milk yield and composition of CG and TGs were subjected to the MANOVA model including the repeated measure. Data were subjected to variance tests using a completely randomized block design (CRD). Group means were compared by Duncan multiple range tests (DMRT) at 5% probability ($\alpha \leq 0.05$). These analyses were carried with statistical packages for the social sciences (M/s IBM India Pvt. Ltd., SPSS v 14.0).

RESULTS AND DISCUSSION

Chemical composition, feed intake and digestibility

Table 1 shows the chemical composition of FMS and CFM. Sorghum grain or AIBP plus urea accounted for 95% of the total ingredient composition and isonitrogenous but, other nutrients were significantly different. The chemical composition of AIBP varies significantly based on its physical form (Rathod and Srinivas, 2018). All the AIBPs are rich in cell walls but their percent is highly variable ($P \leq 0.01$) because of their morphological and milling characteristics. FMS intake between groups was comparable but, CFM intake was significantly different ($P \leq 0.01$). Total diet intake with AIBPs was comparable to sole grain (Singh and Srinivas, 2016) or OSM based CFM (Mohanavel and Srinivas, 2016). DMI efficiency in terms of 4% FCM yield or milk TS was comparable between CG and TGs. Digestibility of CP, celluloses and hemicelluloses was higher ($P < 0.01$) in TGs compared to CG (Table 2). The digestible CP to

Table 1: Chemical composition of roughage and CS.

Parameter	Ragi straw	Concentrate supplement					SEM
		CG	T1	T2	T3	T4	
Dry matter	91.60±0.87	86.78 ^a	98.43 ^b	98.6 ^b	97.85 ^b	98.34 ^b	0.59 ^{**}
Organic matter	93.21±0.08	91.28 ^b	93.56 ^c	92.26 ^d	91.32 ^d	93.14 ^a	0.49 ^{**}
Crude protein	3.81±0.21	18.46	18.65	18.76	18.50	18.60	0.27
Crude fat	1.64±0.08	4.37 ^b	4.2 ^b	1.66 ^a	5.57 ^c	1.13 ^a	0.28 ^{**}
Total carbohydrates	6.78±0.13	68.45 ^c	70.72 ^b	71.84 ^a	67.25 ^a	73.42 ^d	0.49 ^{**}
Total ash	87.75±0.08	8.72 ^d	6.43 ^a	7.74 ^c	8.68 ^d	6.85 ^b	0.12 ^{**}
Acid Insoluble Ash	2.39±0.10	1.72 ^c	0.04 ^a	0.48 ^b	0.14 ^a	0.44 ^b	0.06 ^{**}
Cell contents	36.18±2.70	57.00 ^d	46.42 ^b	52.5 ^c	40.49 ^a	42.42 ^a	0.64 ^{**}
NDF	63.82±2.70	43.00 ^a	53.58 ^c	47.5 ^b	59.51 ^d	57.58 ^d	0.64 ^{**}
ADF	36.43±1.83	15.73 ^a	22.31 ^b	34.19 ^c	53.58 ^d	53.65 ^d	1.08 ^{**}
Hemicelluloses	27.39±3.19	27.27 ^c	31.27 ^d	13.31 ^b	5.93 ^a	3.93 ^a	1.14 ^{**}
Cellulose	30.44±1.67	20.27 ^d	9.48 ^b	10.24 ^b	16.85 ^c	6.85 ^a	0.67 ^{**}
NFC	23.93±2.73	29.53 ^e	17.21 ^b	21.64 ^d	9.02 ^a	19.37 ^c	0.66 ^{**}
Gross energy	3.81±0.01	3.87 ^b	3.95 ^c	3.77 ^a	3.93 ^c	3.78 ^a	0.02 ^{**}

Values bearing different alpha-superscripts in a row indicates significant difference between concentrate supplements * $P \leq 0.05$ and ** $P \leq 0.01$.

Table 2: Nutrient intake and digestibility.

Parameter	Control	T1	T2	T3	T4	SEM
Metabolic body weight (kg)	80.71	78.73	77.68	77.43	77.57	4.35
Ragi straw intake (kg/d)	5.66	5.61	5.63	5.62	6.38	0.31
Concentrate intake (kg/d)**	1.68 ^c	1.63 ^a	2.16 ^e	1.81 ^d	1.65 ^b	0.01
Total DM intake (kg/d)	6.40	6.28	7.11	6.67	7.27	0.37
(g/kg W ^{0.75})	80.11	80.65	92.24	86.59	93.87	5.74
Feed efficiency						
g FCM/kg DMI	541	604	553	417	567	78
g milk solids/kg DDMI	122	131	110	86	123	18
Nutrient digestibility (%)						
DM	58.76	59.63	62.86	64.17	58.45	2.09
OM	60.58	61.67	64.88	65.65	64.45	2.29
CP**	70.92 ^a	85.38 ^b	84.46 ^b	85.15 ^b	71.95 ^a	2.01
EE*	62.89 ^b	53.94 ^{ab}	79.62 ^c	59.85 ^{ab}	47.51 ^a	4.35
TCHO	59.69	59.69	62.38	64.06	64.18	2.56
Total ash	36.35	32.28	36.88	45.88	22.11	2.86
Energy	65.66	67.33	71.82	71.36	69.59	2.38
Cell contents	61.02	62.30	66.71	58.57	58.28	1.85
NFC	61.32	63.42	68.34	62.91	65.30	2.48
NDF	57.02	57.80	60.04	67.71	58.50	1.85
ADF	54.89	45.50	49.24	60.01	51.90	4.10
Hemicelluloses**	59.20 ^a	70.98 ^b	75.95 ^{bc}	81.63 ^c	70.06 ^b	2.17
Celluloses**	80.05 ^a	84.25 ^a	89.94 ^b	89.33 ^b	91.56 ^b	1.59

Values bearing different superscripts of a parameter in row indicated significant difference. * $p \leq 0.05$ and ** $p \leq 0.01$.

energy (PE) ratios of CG and T4 were 11.3 and 12.1, respectively in contrast to 7 or 8 in T1, T2 and T3. The wider PE ratio may decrease the efficiency of urea-ammonia assimilation by rumen microbes. The digestible EE intake in T2 and T4 was 118 and 112 g/d in contrast to 150 to 180 g/d in CG, T1 and T3 ($P \leq 0.05$). The hemicelluloses and celluloses digestibility were significantly higher in AIBPs than sorghum grain. According to Oba and Allen (1999), a unit

increase in NDF digestibility would increase 170 g/d increase in DMI and 250 g/d increase in milk yield.

Energy partition and MBP production

ME intake was 13 to 16 Mcal/d, respectively and comparable ($P = 0.14$) in different groups between CG and TGs (Table 3). Energy retained (ER) was also comparable ($P = 0.11$) between groups. ER to heat increment ($P = 0.19$) ratio was

Table 3: Energy (MJ) partitioning.

Parameter	Control	T1	T3	T2	T4	SEM
DE, Mcal	16.05	16.22	19.35	18.24	19.34	1.23
ME, Mcal	13.06	13.12	15.73	14.87	15.82	1.04
Q-value, ME/GE	0.53	0.54	0.58	0.58	0.57	0.02
Energy retention, Mcal	2.60	2.69	4.20	3.88	4.20	0.59
Energy efficiency,%	19.80	20.46	26.59	25.87	25.38	2.40
Heat increment, Mcal	10.45	10.44	11.52	10.99	11.62	0.56

Table 4: Urinary purine derivatives and rumen microbial protein production.

Parameter	Control	T1	T3	T2	T4	SEM
Purine derivatives, mmol/l urine**	17.66 ^b	9.12 ^b	9.32 ^a	8.46 ^a	17.37 ^b	1.08
Creatinine (mmol/l of urine)	2.82	3.73	4.37	5.01	3.53	0.57
PD: Creatinine (PDC) ratio**	6.51 ^b	2.58 ^a	2.16 ^a	1.81 ^a	5.38 ^b	0.66
PDC index**	516.35 ^b	197.91 ^a	166.39 ^a	138.49 ^a	423.07 ^b	51.90
PD Production (g/d)**	309.12 ^b	94.70 ^a	73.87 ^a	55.12 ^a	247.47 ^b	15.58
MBP flow to duodenum (g/d)**	224.74 ^b	68.85 ^a	53.71 ^a	40.07 ^a	179.92 ^b	13.40
MBP efficiency (gN/kg DOMI)**	63.25 ^b	19.29 ^a	12.59 ^a	9.83 ^a	41.38 ^b	6.17
MBP efficiency (gN/MJ ME)**	17.37 ^b	5.29 ^a	3.42 ^a	2.67 ^a	11.50 ^b	1.71

Values bearing different superscripts of a parameter in a row indicated significant difference. * $P \leq 0.05$ and ** $P \leq 0.01$.

Table 5: Milk yield and composition.

Treat.	Week							Mean
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	
4% FCM yield (kg/d)								
Control	4.14	3.96	3.65	3.17	3.38	3.11	2.83	3.47
T1	4.13	4.08	4.11	3.90	3.85	3.54	3.32	3.85
T2	3.83	3.95	3.89	3.95	3.90	3.88	3.89	3.90
T3	3.12	2.99	2.95	2.83	2.69	2.46	2.39	2.78
T4	4.24	4.07	4.41	4.40	4.37	4.30	4.37	3.90
SEM	.62	0.60	0.60	0.60	0.63	0.61	0.60	0.02
Total solids (%)								
Control	12.46	12.50	12.56	12.57	12.58	12.59	12.65	12.56 ^a
T1	12.79	13.01	13.04	12.94	13.08	13.07	13.16	13.01 ^c
T2	12.94	13.09	13.09	13.04	13.09	13.02	13.08	13.05 ^c
T3	12.38	12.48	12.66	12.59	12.65	12.65	12.70	12.59 ^b
T4	13.38	13.55	13.58	13.57	13.61	13.55	13.54	13.54 ^d
SEM	0.32	0.33	0.32	0.32	0.32	0.35	0.35	0.04 ^{**}
Fat (%)								
Control	3.52	3.56	3.64	3.64	3.67	3.69	3.71	3.63 ^c
T1	3.85	4.04	4.10	3.96	4.10	4.12	4.16	3.47 ^b
T2	4.09	4.18	4.19	4.18	4.21	4.15	4.20	3.18 ^a
T3	3.53	3.56	3.79	3.72	3.77	3.80	3.81	3.71 ^d
T4	4.45	4.63	4.64	4.64	4.67	4.61	4.61	3.45 ^b
SEM	0.33	0.33	0.34	0.33	0.34	0.36	0.34	0.04 ^{**}
Solids non fat (%)								
Control	8.95	8.94	8.93	8.93	8.91	8.91	8.93	8.93
T1	8.94	8.97	8.95	8.98	8.98	8.95	9.00	8.97
T2	8.85	8.91	8.90	8.86	8.88	8.87	8.88	8.88
T3	8.85	8.92	8.87	8.87	8.89	8.85	8.89	8.87
T4	8.93	8.92	8.94	8.93	8.94	8.95	8.93	8.93
SEM	0.30	0.28	0.37	0.36	0.03	0.04	0.03	0.03

Values bearing different superscripts of a parameter in a column indicated significant difference. * $p \leq 0.05$ and ** $p \leq 0.01$.

25:75 in CG and T1, while 35:65 in T2, T3 and T4, respectively. All the energy terms were comparable between CG and TGs hence, AIBPs were equally good sources of energy for dairy cows. Total PD excreted in the urine of CG and T4 was 18 mM/L (Table 4). This was two folds higher than other TGs ($P \leq 0.01$). Although creatinine excretion in urine was comparable ($P = 0.11$) between groups, the ratio between PD and creatinine ($P \leq 0.01$) and PDC index were significantly different between groups ($P \leq 0.01$). The duodenal flow of MBP was higher in CG followed by T4 but least in T1, T2 and T3 ($P \leq 0.01$). Cereal and pulses grit composition resembles whole grain because they were broken fragments of whole grain while milling (Rathod and Srinivas, 2018). This indicated that the energy available in the AIBP may equally cater to the host cows like grains but not the requirements of rumen microbes for their growth. This means that the type of carbohydrate energy source in AIBP was not suitable for the quantitative improvement of rumen MBP. Non-fibrous carbohydrates (NFC) are important for the rumen MBP and it was optimum on balanced rations (Sherasia *et al.*, 2016). Rumen MBP has been observed to be higher when NFC in either OSM or grains was $\geq 30\%$ and decreased when NFC was below 20% (Mohanavel and Srinivas, 2016, Singh and Srinivas, 2016). In the absence of adequate rumen MBP production, the metabolic and health consequences are yet to be known and probably, first to document the adversity of AIBP on rumen MBP.

Milk yield and composition

Repeat measure analysis of total milk yield or 4% FCM milk yield indicated a significant difference ($P \leq 0.01$) between weeks but, non-significant ($P = 0.13$) between groups (Table 5). The impact of AIBP on milk yield and composition is well documented and our results were also phenomenal but, the distinctive observation was reduced coefficient of variation (CV) in daily milk yield from 25 to below 3% ($P \leq 0.01$). This could be possible on AIBP because of their slower rate of fermentation than grains that improve the spatial distribution of nutrients in the system over time (Rathod and Srinivas, 2018). There was no significant difference in the milk composition yet the mean milk TS and fat at the end of the experimental period were significantly improved in TGs than CG. The efficiency of 4% FCM yield or TS in milk was comparable between CG and TGs. Pre-mix of feed additives reported to have impact on milk composition (Lokesha and Srinivas, 2018). Mean milk protein was 3.34%, 3.51%, 3.44%, 3.23% and 3.58%, respectively in CG, T1, T2, T3 and T4 ($P \leq 0.01$). Milk protein resembles the amino acid composition of rumen MBP however, its *de novo* synthesis in mammary gland play important role. Hence, milk protein% was independent of rumen MBP but influenced by the dietary protein source significantly. Compared to grains, higher milk protein% was higher in bran and grit or grit in combination with husk but lest on husk alone.

CONCLUSION

The sole feeding of AIBP as concentrate feedstuffs would

deter the rumen MBP production but not the milk yield or composition because of limitations in NFC, particularly starch availability. The hemicelluloses present in AIBP appear to support milk yield but had limited influence as a source of energy to the rumen microbial growth. Hence, the addition of limited quantities of starch sources can substantiate the inadequacy of AIBP to promote rumen MBP when used in dairy rations.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-NDRI, Karnal, Haryana (India) for the financial assistance and for extending the facilities. The authors declare that they have no conflict of interest for the products or outcomes.

Conflict of interest: None.

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