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Research Article

EFFECT OF SOLID STATE FERMENTATION (SSF) BIOMASS ON NUTRIENT DIGESTIBILITY, RUMEN PARAMETERS AND MICROBIAL PROTEIN SYNTHESIS IN CROSSBRED HEIFERS

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Abstract: The present study was carried out on fifteen crossbred heifers to evaluate the effect of solid state fermentation (SSF) bio-mass supplementation on nutrient digestibility, rumen parameters and microbial protein synthesis in crossbred heifers. For that, fifteen crossbred heifers were randomly divided into three group of five each based on body weight and were individually fed for the 98 days. The heifers were fed total mixed ration (TMR) without SSF (T_1), TMR with 3% SSF biomass (T_2) and TMR having 10% reduction in protein and energy with 3% SSF biomass (T_3). The results revealed that the digestibility coefficient of DM, EE, NFE, NDF and ADF were not differ by the treatment group. However, the crude protein digestibility (%) of T_2 group was significantly ($P<0.05$) higher. The crude fibre digestibility (%) in treatment group were significantly different from each other. The rumen parameters were not affected among the groups. The microbial protein synthesis in-creased non-significantly in T_2 group. Hence, inclusion of SSF biomass in TMR based diet helped in improving crude pro-tein and fibre digestibility and numerically increased the microbial protein synthesis in heifers.

Keywords: *SSF biomass, Digestibility, Microbial protein, Rumen parameters, Heifers*

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Introduction

Due to the presence of lignocellulose linkages, high amounts of silica, and anti-nutritional elements, agricultural by-products and cereal crop wastes normally make up the majority of nursing ruminant rations, which are low in nutrients and less digestible. This will not only reduce output potential but also has an impact on farmer profitability. In such conditions use of feed additives appears to be an attractive solution. Ruminants have a wide range of microorganisms that can consume such feeds, but proper modification of the ruminant ecology can boost feed digestibility and economic returns even more. In such cases, enzymes, probiotics, prebiotics, and nutraceuticals have been reported to be effective [11].

Solid state fermentation (SSF) is defined as fermentation involving solid in absence (or near absence) of free water. SSF encouraged the growth of micro-organisms in nature on moist solids and has been created to be blameable for the beginning of fermentation technique in earliest time. It provides various scope in processing of agro industrial residues [10]. SSF holds remarkable potential for the production of enzyme by microbial flora. This procedure is particularly intriguing since it produces a crude fermented product that can be used as a direct source of enzymes. Preferably, almost all well-known microbial enzymes can be yields under SSF system. A huge amount of microorganisms, including bacteria, yeast and fungi yield different groups of enzymes, but fungi are the furthermost suitable organism [6].

Materials and Methods

Animals, feeding, management and dietary treatments

Fifteen crossbred heifers were randomly divided into three equal groups of five in each group, based on body weight. The present study was conducted at Animal Nutrition Research Station, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat during the year 2019 for the period of 98 days. The permission for animal experiment was granted by Institutional Animal Ethics Committee (IAEC 2019/ANRS/296).

All the experimental heifers were fed on TMR in mash form with or without SSF to meet their nutrients needs as per standards [8]. The experimental heifers were housed in sheds with proper ventilation, flooring and tying arrangements. Individual feeding of all the heifers was followed. The crossbred heifers in control group (T_1) were fed total mixed ration containing 50 % Jowar straw without SSF bio-mass, while in group T_2 were fed TMR containing jowar straw and 3 % SSF biomass whereas, animals in group T_3 were fed wheat straw with 10% reduction in protein and energy and 3% SSF biomass. The SSF on jowar straw was prepared by Department of Microbiology, Gujarat Vidyapith, Sadra with a culture of Aspergillus spp., having activity of various enzymes i.e., carboxy methyl cellulase (CMCase) (124 U/g), filter paperase (FPase) (27.85 U/g), xylanase (306 U/g), laccase (377 U/g), manganese peroxidase (MnP) (330 U/g) and lignin peroxidase (LiP) (307 U/g) assessed by using enzymatic assay technique.

The experimental heifers were let loose daily for exercise (except during the period of digestion trial) in an open paddock, for two hours in the morning and one hour in the afternoon under controlled conditions during which they had free access to fresh, wholesome drink-ing water.

Digestion trial

At the end of the experiment the digestion trial was conducted having 6 days of collection period. The daily feed intake, feed refusal and daily faeces voided were recorded during the digestion trial and the representative samples of faeces, feed offered and left over were taken for its proximate [1] and fibre fractions [19] analyses. The average digestibility of nutrients was calculated for whole ration using the data of the digestion trial. To calculate the daily nutrients intake by each heifer, representative samples of feeds were collected for proximate analyses [1].

Rumen parameters

The samples of rumen liquor were collected from individual heifers at 0 (before feeding), 3 and 6 h post feeding during the digestion trial.

The pH of strained rumen liquor (SRL) was determined immediately after collection using portable digital pH meter. After pH de-termination, 1.0 mL of saturated $HgCl_2$ solution was added to each sample to stop microbial activity in the SRL. The samples of SRL were analyzed for ammonia-N, total-N and various N fractions. The total volatile fatty acids (TVFAs) concentration was evaluated by the steam distillation technique using Markham micro-distillation apparatus.

Microbial protein synthesis

Urine samples (100 mL) were collected from individual heifers for three consecutive days and assayed for allantoin, uric acid and creatinine [20]. Purine derivatives (PD) were assessed by spot sample test based on the principle that excretion of creatinine is constant throughout a day, hence, creatinine was used as an internal marker for quantification of PD [5]. Daily excretion of creatinine was considered as 0.98 mmol/kg W0.75 and microbial N supply was calculated from the daily urinary PD excreted [7].

Table-1 Ingredient and their chemical composition (%) of total mixed rations (TMRs) offered to experimental heifers

Ingredient	T_1	T_2	T_3
Jowar hay	50	50	25
Soybean meal	10	10	8.5
Maize	12.5	12.5	3
DORB	15.5	15.5	28.5
Wheat straw	0	0	25
Molasses	10	10	8
Mineral mixture	1	1	1
Common salt	1	1	1
Chemical composition (% on DM basis)			
Crude protein	13.66	13.66	12.04
Ether extract	1.35	1.35	1.35
Crude fibre	29.41	29.41	27.95
Nitrogen-free extract	47.08	47.08	45.82
Total ash	8.5	8.5	12.85
Organic matter	91.5	91.5	87.15
Neutral detergent fibre	66.97	66.97	63.92
Acid detergent fibre	30.4	30.4	29.52
Calcium	2.85	2.85	2.75
Phosphorus	0.09	0.09	0.09

Table-2 Effect SSF biomass on nutrient intake in heifers

Attributes	Groups		
	T_1	T_2	T_3
DM (g/d)	4602.34 ± 225.25	4683.60 ± 231.59	4595.14 ± 211.91
CP (g/d)	628^{a}.67 ± 30.76	639^{a}.77 ± 31.63	553^{b}.25 ± 25.51
DCP (g/d)	361.49^{b} ± 17.59	402.42^{a} ± 19.89	319.78^{c} ± 14.74
TDN (g/d)	2444.30^{a} ± 119.63	2484.18^{a} ± 122.83	2127.09^{b} ± 98.09

^{abc} Means with different superscripts within a row differ significantly (P<0.05)

Table-3 Effect SSF biomass on digestibility coefficient (%) in heifers

Attributes	Groups		
	T_1	T_2	T_3
DM	59.17 ± 2.25	59.17 ± 2.02	55.21 ± 1.05
CP	57.59^{b}± 0.77	62.94^{a}± 0.56	57.85^{b} ± 1.97
CF	63.29^{ab} ± 2.07	69.27^{a} ± 2.01	59.75^{b} ± 1.87
EE	60.02 ± 1.95	55.61 ± 4.38	56.72 ± 6.19
NFE	52.69 ± 2.34	47.54 ± 3.03	45.62 ± 1.56
NDF	63.44^{a} ± 2.10	63.47^{a} ± 2.53	54.89^{b} ± 1.27
ADF	49.15 ± 3.45	48.75 ± 3.55	44.34 ± 1.38

^{abc} Means with different superscripts within a row differ significantly (P<0.05)

Table-4 Effect SSF biomass on rumen parameters in heifers

Attributes	Groups		
	T_1	T_2	T_3
pH	6.97	6.92	7
Total N (mg/dL)	52.5	54.36	54.13
Ammonia- N (mg/dL)	20.88	21.73	17.5
NPN (mg/dL)	30.56	28.46	27.76
Soluble N (mg/dL)	26.48^{a}	20.65^{b}	16.45^{c}
TVFAs (mmol/dL)	14.26^{a}	15.45^{a}	12.85^{b}

^{abc} Means with different superscripts within a row differ significantly (P<0.05)

Table-5 Effect SSF biomass on microbial protein synthesis in heifers

Attributes	Groups		
	T_1	T_2	T_3
Allantoin (mmol/L)	0.29 ± 0.04	0.31 ± 0.02	0.23 ± 0.03
Uric acid (mmol/L)	1.17 ± 0.18	1.26 ± 0.08	1.34 ± 0.03
Creatinine (mmol/L)	2.97 ± 0.26	3.22 ± 0.24	3.36 ± 0.35
Total PD excreted (mmol/d)	23.84 ± 3.35	25.07 ± 2.35	24.50 ± 3.26
PDC index	24.33 ± 3.42	25.58 ± 2.39	25.00 ± 3.33
Absorbed purine (mmol/d)	19.49 ± 3.91	20.58 ± 2.57	20.09 ± 3.90
Intestinal flow of microbial nitrogen supply (g/d)	14.84 ± 2.84	14.96 ± 1.86	14.60 ± 2.83

^{abc} Means with different superscripts within a row differ significantly (P<0.05)

Results and Discussion

Nutrient digestibility

The ingredient and chemical composition of TMRs used for feeding the experimental heifers in all three treatment groups has been presented in [Table-1]. The solid state fermented biomass contained 7.74, 3.09, 35.79, 67.74 and 40.60% CP, EE, CF, NDF and ADF on DM basis, respectively.

The digestibility coefficients of TMRs obtained for different nutrients in the control and treatment groups are presented [Table-3]. The average DM digestibility coefficients of experimental heifers under T_1, T_2 and T_3 groups were 59.17 ± 2.25, 59.17 ± 2.02 and 55.21 ± 1.05 per cent, respectively. The DMD of heifers in control and SSF biomass supplemented groups were not differ from each other. Similar findings were reported by Shekhar, *et al*., (2010) [14] & Awawdeh and Obeidat (2011) [2]. The average CP digestibility coefficient of experimental crossbred heifers under T_1, T_2 and T_3 groups were 57.59b ± 0.77, 62.94a ± 0.56 and 57.85b ± 1.97 %, respectively. The CP digestibility of T_2 group was significantly (P<0.05) higher than T_1 and T_3 groups. Chaudhari (2018) [4], Pinos-Rodríguez, *et al*., (2002) [13] and Singh and Das (2008) [16] indicated similar trend to the present findings. The average EE digestibility coefficient in T_1, T_2 and T_3 groups were 60.02 ± 1.95, 55.61 ± 4.38 and 56.72 ± 6.19 %, respectively. The statistical analysis revealed non-significant difference among the groups. This agreed with Shekhar, *et al.,* (2010) [14]. The average values for CF digestibility coefficient of experimental crossbred heifers in T_1, T_2 and T_3 groups were 63.29ab ± 2.07, 69.27a ± 2.01 and 59.75b ± 1.87 %, respectively. The T_1, T_2 and T_3 groups were significantly differ from each other for CF digestibility. Similar to our findings, Sherasia, *et al*., (2018) [15] also reported highly significant (P<0.01) difference in digestibility coefficient of CF in cattle fed SSF biomass. The average values for NFE digestibility coefficient in T_1, T_2 and T_3 groups were 52.69 ± 2.34, 47.54 ± 3.03 and 45.62 ± 1.56 %, respectively. The treatment groups did not differ statistically (P>0.05) from each other. This is in agreement with findings of Chaudhari (2018) [4]. The average values for digestibility coefficient of NDF in T_1, T_2 and T_3 groups were 63.44a ± 2.10, 63.47a ± 2.53 and 54.89b ± 1.27 %, respectively. The NDF digestibility of T_1 and T_2 groups were in-creased significantly (P<0.05) as compare to T_3 group. In contrast to the present findings, non-significant effect on NDF digestibility was reported by [4] in calves fed SSF biomass at 3 % level in TMR. The average values for ADF digestibility coefficient in T_1, T_2 and T_3 groups were 49.15 ± 3.45, 48.75 ± 3.55 and 44.34 ± 1.38 %, respectively. The treatment groups did not differ statistically (P>0.05). This is in agreement with findings of Sherasia, *et al*., (2018) [15].

Rumen parameters

The average values for rumen parameters of crossbred heifers during 0 hr (pre-feeding) and different hours of post feeding are summa-rized [Table-4]. The average pH values in SRL of T_1, T_2 and T_3 groups were 6.97, 6.92 and 7.00, respectively. The difference for hours of feeding for SRL pH were significant (P<0.05). But the difference for treatments along with treatment period interactions were non-significant (P>0.05). Similar to present findings, [3] and [9] observed the same. The average TVFA concentration in SRL was 14.26a, 15.45a and 12.85b mM/dl in T_1, T_2 and T_3 groups, respectively. The experimental crossbred heifers under treatment groups showed significant increase in T_1 and T_2 as compared to T_3. But periodical changes were non-significant (P<0.05). The statistical differences for treatment and period interactions were non-significant (P>0.05).

Similar to present findings, Chaudhari (2018) [4] & Singh and Das (2009) [17] reported significantly higher (P<0.05) TVFA values in enzyme treated group. The average total-N concentration (mg/dl) in SRL was 52.50, 54.36 and 54.13 under T_1, T_2 and T_3 groups, respectively. The value of total-N concentration under all groups was non-significant (P<0.05). The differences for hourly changes in total-N concentrations were significant (P<0.05). However, interaction between treatment and period was non-significant (P>0.05). The present findings agreements with Oza, *et al*., (2015) [9]. The average SRL ammonia-N concentration of heifers were 20.88, 21.73 and 17.50 mg/dl under T_1, T_2 and T_3 groups, respectively. These values for ammonia-N concentrations were non- significant (P>0.05) under all the groups. Hourly changes in ammonia-N concentrations were significant (P<0.05). However, the differences for interaction between treatment and period were non-significant (P>0.05). Similar effects were also noted by Singh and Das (2009) [17]. The average concentration of NPN in SRL was 30.56, 28.46 and 27.76 mg/dl under T_1, T_2 and T_3, respectively. The concentration of NPN were similar (P>0.05) in all the groups. Hourly changes in NPN concentration along with the treatment and period interactions were also non-significant (P>0.05). In contrast, Patel (2012) [12] reported that diet containing solid state fermentation (SSF) had highly significant (P<0.01) effect observed in treatment group. The average soluble nitrogen concentrations in SRL were 26.48a, 20.65b and 16.45c mg/dl in T_1, T_2 and T_3 groups, respectively. The periodical differences were non-significant (P<0.05), however, the treatment differences were significant (P<0.05). Statistical differences for treatment and period interactions were non-significant (P>0.05).

Microbial protein synthesis

The microbial protein was calculated on the basis of spectrophotometric analysis of purine derivatives (uric acid, allantoin, and creatinine) from urine samples of individual heifers [Table-5]. The average rumen microbial protein synthesis in T_1, T_2 and T_3 groups was 14.84, 14.96 and 14.60 g/day, respectively. The non-significant difference observed among all the groups. Results indicated that SSF containing TMRs have non-significant improvement on rumen microbial protein synthesis. In contrast, Chaudhari (2018) [4] reported 37.44% higher microbial protein synthesis (p<0.05) in crossbred calves fed SSF biomass in TMR.

Conclusion

The supplementation of solid state fermentation (SSF) biomass @ 3 % in jowar hay based TMR revealed that CP and CF digestibility were improved by 9.20 and 9.44%, respectively, in T_2 as compared to the T_1 and T_3 groups. The values for rumen parameters *viz*. pH, TVFA, ammonical N, NPN, total N and soluble N were found for treatment and period interactions were non-significant in all groups. The rumen microbial protein synthesis increased non-significantly in T_2 group as compared to control group.

Application of research: This research can be applied by farmers to improve profitability by using SSF biomass in feed.

Research Category: Feed additive

Abbreviations: DM: Dry matter, CP: Crude protein, CF: Crude fibre
EE: Ether extract, NFE: Nitrogen free extract, NDF: Neutral detergent fibre
ADF: Acid detergent fibre, SRL: Strained rumen liquor, PD: Purine derivatives
NPN: Non protein nitrogen, DORB: De-oiled rice bran,
TDN: Total digestible nutrient, DCP: Digestible crude protein

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