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An *In vitro* Approach to Optimize Levels of *Moringa oleifera* Meal Incorporation in Total Mixed Ration for Crossbred Heifer Calves

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ABSTRACT

An *in vitro* experimentation outside living animals to evaluate feed/fodders on digestibility and gas production is cheap, easy and having more control condition as compared to an in vivo evaluation on livestock which is costly and time-consuming event. The incorporation of *Moringa oleifera* meals @ 2.5– 22.5% with increment of 2.5% in total mixed ration-TMR replacing concentrate mixture (50%) was assessed on *in vitro* digestibility, total gas production (TGP) and metabolizable energy (ME) content for growing crossbred heifer calves. The roughage part (50%) of TMR was hybrid napier (15%) and ground nut straw (35%). All these ingredients were oven dried for twenty four hour at 70°C and finely ground in hammer mill using 1 mm sieve. 500 mg of each TMR were incubated at 39±1°C for 48 hours in triplicate along with blank without TMR. The results of experiment revealed that incorporation of *Moringa oleifera* meal @ 5.0% with increment levels of 2.5% and higher levels up to 22.5% to replace high protein concentrate mixture (50%) in total mixed ration containing 50% roughage (15% hybrid napier and 35% ground nut straw) has improved ($p<0.05$) *in vitro* dry matter digestibility (60.879±0.251 to 64.678±0.679%), *in vitro* gas production (81.000±9.018 to 95.667±1.764 ml/500 mg TMR) and ME content (2.356±0.068 to 3.223±0.057 Mcal ME kg^{-1} TMR) of total mixed ration. The levels 5% and above up to 22.5% with increment of 2.5% level in total mixed ration replacing concentrate mixture was found suitable for *in vivo* growth study of crossbred heifer calves.

KEYWORDS: Crossbred heifer, digestibility, gas production, *in vitro, Moringa*, ME

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1. INTRODUCTION

Feed and fodder are key nutritional input to supply carbohydrate, protein, vitamins and mineral for efficient performance of growing dairy animals (Pulina et al., 2020). India faces a net deficit of green fodder (35.6%), dry crop residues (10.95%) and concentrate feed ingredients (44%). The feeding systems based on forage are mandatory to lower the livestock rearing cost as the feed account 60-70% of the total livestock rearing cost (Anonymoys, 2013). The cheap and easily available unconventional feedstuffs are imperative to livestock production system (Oduro et al., 2008). Moringa is well known to have nutritional and pharmacological properties (Soliva et al., 2005). Moringa leaves are non-expensive, easily available and unconventional source of nutrients (Dong et al., 2019) and are abundant in protein, amino acids, fatty acids, minerals, vitamins, calcium, potassium which are the basic to growth of animal body (Deshmukh, 2014) along with various phenolic and oxycaroteniod compounds. The high biological value of nutrients of leaves have potential for adoption as feed for ruminant (Pradhan, 2016). This high valued Leaves' protein and other nutrients need to evaluate for growth of dairy heifer calves replacing the concentrate mixture. An *in vitro* experimentation outside living animals to evaluate *Moringa oleifera* meal incorporation in total mixed ration on digestibility is cheap, short duration and having more control condition (Makkar, 2004) as compared to an in vivo evaluation on livestock which is costly and time-consuming event.

It was hypothesized that incorporation of *Moringa oleifera* meals in total mixed ration will improve in vitro fermentation kinetics. The study was planned to assess the effect of incorporation of various levels of *Moringa oleifera* meals in TMR on in vitro digestibility, total gas production (TGP) and metabolizable energy (ME) content for growing crossbred heifer calves.

2. MATERIALS AND METHODS

The study was undertaken at Animal Nutrition Research Department, College of Veterinary Science and A. H., Anand Agricultural University, Anand, Gujrat, India during 2020–2021 after an ethical approval (LRS/322/2020) from the Institutional Animal Ethics Committee of College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand.

Green hybrid napier, *Moringa oleifera* fodder, groundnut straw and compound concentrate mixture were procured from Livestock Research Station, Anand Agricultural University, Anand was used for this experiment. Green hybrid napier was chopped 5-6 cm length and sun dried on cemented floor for three days. PKM 1 variety of *Moringa oleifera* fodder was harvested after 75 days showing at 1.5 feet above the ground level was used for study. Moringa fodder was sun dried for 3 days on cemented yard. Leaves and stems were separated after drying moisture level near 10 %. Leaves and soft twigs were used for this study. The soft twigs of Moringa fodder were ground using grinder with 2 mm sieve. Eighty four percent leaves and sixteen percent soft twigs of *Moringa oleifera* was mixed to prepare *Moringa oleifera* meal (MOM). After sun drying of green ingredients, all these ingredients were oven dried for twenty-four hours at 70°C and finely ground in hammer mill using 1 mm sieve. These ingredients were mixed in ratios shown in Table 1 to prepare different total mixed ration (TMR). The TMR was prepared to meet nutritional requirement of crossbred dairy heifer calves weighing 100 kg and growing at 700 g day^{-1} as per NRC (2001) standard. The calculated nutritional values of TMRs was 17.46±0.004% crude protein (CP), 2.54±0.005 Mcal ME kg^{-1} DM and 65.43±0.225% TDN.

Table 1: Levels of various ingredients (%) in different TMR

Treatment	CCM	MOM	HN	GNS
T_1	50.0	0	15	35
T_2	47.5	2.5	15	35
T_3	45.0	5.0	15	35
T_4	42.5	7.5	15	35
T_5	40.0	10.0	15	35
T_6	37.5	12.5	15	35
T_7	35.0	15.0	15	35
T_8	32.5	17.5	15	35
T_9	30.0	20.0	15	35
T_{10}	27.5	22.5	15	35

CCM: Compound Concentrate Mixture; MOM: *Moringa oleifera* meal; HN: Hybrid Napier; GNS: Ground nut straw

The ingredients were analyzed for proximate constituents (Anonymous, 1995) and fiber fractions (Van Soest, 1991) and that of different TMRs were calculated based on actual parameters of analysis. The compounded concentrate mixture of TMR replaced with 0, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 and 22.5 percent of MOM, was designated as T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9 and T_{10}, respectively to ascertain the effect on in vitro dry matter digestibility, in vitro TGP, and ME content. Three crossbred calves of same age and breed was selected as donor of rumen inoculums for in vitro study. The crossbred heifer calves used to collect rumen liquor, were fed to meet nutrients requirement as per NRC (2001) with free access to clean and wholesome potable water. Rumen liquor was collected using negative pressure pump with stomach tube from three crossbred heifer calves. The rumen liquor from three crossbred heifer calves was strained through four-layer muslin cloth and mixed. This fluid was termed strained rumen liquor (SRL). 500 mg of each TMR with various levels of compounded concentrate mixture and MOM were incubated at 39±1°C for 48 h in a shaker twin water bath with 40 ml of fresh McDougall buffer and 10 ml SRL as described by Menke et al. (1979) in triplicate. Fresh macro and micro buffer solutions to be use in incubation was prepared and kept in incubator at 39 ^{0}C. After incubation of 48 hours, the content of each syringe was filtered through dried and pre-weighed Gooch crucible, which was again dried and weighed. The blank without TMR also incubated in triplicate to arrive at net gas production. Gas production was measured at 1, 2, 3, 4, 5, 8, 10, 12, 20, 24, 26, 30, 44 and 48 hours after incubation and line chart plotted. The metabolizable energy (ME) content of TMR was calculated as per Elghandour, (2015) and the metabolizable energy content of TMR was converted in Mega-calorie (Mcal).

ME (MJ/kg DM)=2.20+0.136 Gp+0.057 CP%, (R^2=0.94)

Where, CP is crude protein% and Gp is ml of net gas production from 200 mg dry sample.

The digestibility of dry matter, TGP and ME were analyzed (Snedecor and Cochran, 1994) using online WASP- Web Agri State Package software developed by Central Coastal Agricultural Research Institute, Goa.

3. RESULTS AND DISCUSSION

The proximate and fiber fractions composition of ingredients and TMR used in experiment are presented in Table 2. The total gas production, in vitro dry matter digestibility (IVDMD) and metabolizable energy of different TMRs are presented in Table 3. An average content of crude protein (CP), ether extract (EE), crude fibre (CF), nitrogen free extract (NFE), total ash (TA), neutral detergent fibre (NDF), acid detergent fibre (ADF) and total digestible nutrients was 17.46±0.004, 3.22±0.006, 24.53±0.102, 43.98±0.032, 10.87±0.144, 50.14±0.266, 39.82±0.158 and 65.43±0.225%, respectively. An average metabolizable energy (ME) of TMRs was 2.54±0.005 Mcal/kg DM. Dey et al. (2014) had reported comparable crude protein (26.34±0.67%) of Moringa leaves. However, Nouala et al. (2006) had reported slight lower CP (23.27%), NDF (18.74%) and ADF (16.07%) of *Moringa oleifera* leaves. Seradja et al. (2019) also reported lower crude protein as 20.1, 21.0 and 20.4% of DM of Moringa forage consist of leaves, twigs and new buds harvested at 30, 40 and 50 days after pruning, this variation may be due to different agro-climatic zone and variety of Moringa.

3.1. In vitro total gas production (IVTGP)

An *in vitro* gas production of TMRs incorporated with different levels of MOM was significantly (p<0.05) higher compared to control TMR. However, incorporation of MOM in total mixed ration at 5.0 percent had revealed

Table 2: Average proximate composition, fiber fractions (% on DM basis) and energy value of feeds and fodder

	CP	EE	CF	NFE	TA	NDF	ADF	ME Mcal kg^{-1} DM
CCM	25.48	4.27	16.57	46.34	7.34	39.80	28.32	3.200
MOM	25.32	4.04	12.29	45.01	13.34	28.78	21.52	2.700
HN	7.60	2.44	34.43	45.05	10.48	68.08	52.87	2.000
GNS	10.29	2.12	33.04	40.58	13.97	60.68	52.88	1.900
T_1	17.48	3.24	25.01	44.13	10.19	51.35	40.60	2.565
T_2	17.48	3.25	24.91	44.10	10.34	51.07	40.30	2.560
T_3	17.47	3.23	24.80	44.06	10.49	50.80	40.26	2.555
T_4	17.47	3.23	24.69	44.03	10.64	50.52	40.09	2.550
T_5	17.47	3.22	24.59	44.00	10.79	50.52	39.92	2.545
T_6	17.46	3.21	24.48	43.96	10.94	49.97	39.75	2.540
T_7	17.46	3.21	24.37	43.93	11.09	49.70	39.58	2.535
T_8	17.45	3.20	24.26	43.90	11.24	49.42	39.41	2.530
T_9	17.45	3.20	24.16	43.86	11.39	49.15	39.24	2.525
T_{10}	17.45	3.19	24.05	43.83	11.54	48.87	39.07	2.520

CP: Crude protein; EE: Ether extract; CF: Crude fibre; NFE: Nitrogen free extract; TA: Total ash; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ME: Metabolizable energy; CCM: Compound concentrate mixture; MOM: Moringa oleifera meal; HN: Hybrid Napier; GNS: Ground nut straw

Table 3: Total gas and IVDMD during *in vitro* fermentation and ME content of ration

Treatment	Total gas ml	IVDMD%	ME (Mcal kg^{-1} DM)
T_1	48.333^{e}±1.453	57.459^{bcd}±0.512	1.685^{e}±0.047
T_2	66.333^{d}±8.293	56.898^{cd}±1.322	2.270^{d}±0.270
T_3	93.000^{ab}±1.173	60.879^{abc}±0.251	3.137^{ab}±0.056
T_4	95.667^{a}±1.764	64.678^{a}±0.679	3.223^{a}±0.057
T_5	87.000^{ab}±1.155	62.370^{ab}±1.170	2.942^{ab}±0.038
T_6	69.000^{cd}±2.082	57.842^{bcd}±2.460	2.356^{cd}±0.068
T_7	81.667^{abc}±6.360	53.964^{d}±1.083	2.768^{abc}±0.207
T_8	85.667^{ab}±2.906	56.904^{cd}±1.494	2.898^{ab}±0.094
T_9	84.333^{ab}±0.882	63.223^{a}±4.246	2.855^{ab}±0.029
T_{10}	81.000^{bc}±9.018	61.493^{abc}±0.647	2.746^{bc}±0.293

IVDMD: *In vitro* dry matter digestibility; ME: Metabolizable energy; a,b,c,d,e means with different superscript within column differ significantly ($p<0.05$)

significant (p<0.05) higher IVTGP, and further higher incorporation of MOM in TMR was without improvement in total gas production. Results of *in vitro* fermentation shown significantly (P<0.05) higher total gas production when compounded concentrate mixture was replace with MOM at 5% and higher levels. A gas production characteristic was presented in Figure 1. The lag phase time was about one hour and similar in all the TMRs.

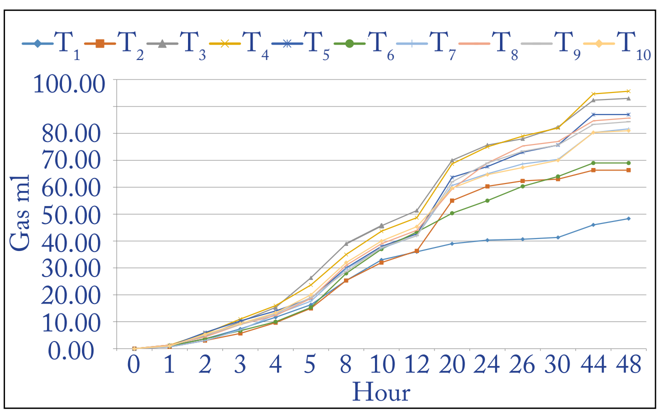

Figure 1: *In vitro* gas production (ml) at different time interval

The Moringa leaves containing feed mixture produce significant effect on fermentation which resulted in higher ($p<0.001$) gas production (up to +2.30%) and the values of gas production were 144.13, 194.13, 149.15, 152.56 and 155.96 ml g^{-1} DM on *in vitro* incubation of *Moringa oleifera* leaves (ML) replacing the wheat straw (WS) @ 0, 100, 5, 10 and 20%, respectively for buffalo as revealed the study of Dey et al. (2014). Similarly, linearly higher ($p<0.0001$) in vitro gas production of feed mixture at 24 hours incubation was noted by Nouala et al. (2006) when concentrate (40%) part replaced with 0, 25, 50 and 100% *Moringa oleifera*, and roughage part of feed mixture was ground nut hay (60%). In contrast to the present study on initiation of gas production, the discrete lag phase reduction (P=0.04) was observed from 1.1 to 0.0 hours when in vitro incubation of alfalfa hay to *Moringa oleifera* forage cut at 40 days after pruning, respectively (Seradja et al., 2019).

In present experiment, higher *in vitro* gas production might be due to higher limiting amino acids like lysine and Methionine (14.06 and 4.97 g kg^{-1} DM, respectively) in the protein of *M. oleifera* leaves (Henuk, 2018) and optimal composition of amino acids with higher digestible protein content (Babiker et al., 2017) which is essential for rumen microbial multiplication and growth. The early gas production was not observed (no leg phase difference) on replacement of compounded concentrate mixture with *Moringa oleifera* meal in present study may be due to high protein concentrate mixture was replace with *Moringa oleifera* meal and both have readily degradable carbohydrate and other nutrients for initiation of microbial activity during substrate fermentation.

3.2. In vitro DM digestibility (IVDMD)

An *in vitro* rumen fermentation data revealed significant (p<0.05) effect of incorporation of MOM in total mixed ration at 7.5% on *in vitro* dry matter digestibility (64.678±0.679%) in comparison to control TMR (57.459±0.512%) and TMR containing 2.5% MOM (56.898±1.322%). Trend on *in vitro* digestibility of dry matter was observed to be variable, however effect of incorporation of MOM @ 7.5 and 20.0% in TMR was significant ($p<0.05$). The replacement of compounded concentrate mixture with MOM at 7.5% in total mixed ration was found beneficial.

An *in vitro* higher ($p<0.001$) true digestibility was observed when feed mixture, (30 and 40% concentrate and 70 and 60% ground nut hay- roughage, respectively) incubated for 24 hours with 50, 75 and 100% concentrate replaced by *Moringa oleifera* as reported by Nouala et al. (2006). The study of Dey et al. (2014) revealed higher ($p<0.001$) true degradability of dry matter (up to +11.47%) on *in vitro* incubation of *Moringa oleifera* leaves replacing the wheat straw (WS) @ 0, 100, 5, 10 and 20%, respectively for buffalo. The true dry matter degradability was 39.82, 86.40, 45.63, 49.58 and 52.72%, respectively. The higher in vitro dry matter degradability in study might be owing to roughage portion was 50% of total mixed rations and improvement in dry matter degradability suggest strong candidate to replace high protein concentrate mixture for growing crossbred heifers. The higher degradability of TMRs with various levels of *Moringa oleifera* meal might be due to higher rumen microbial growth and multiplication as *Moringa oleifera* meal contains higher digestible protein with optimal composition of amino acids (Babiker et al., 2017) and negligible tannin and other anti-nutritive compounds (Makkar and Becker, 1996).

3.3. Metabolizable energy (ME) content of TMR

The ME content of TMR obtained in present experiment was ranges from 1.685±0.512 to 3.223±0.057 Mega calories per kilogram of TMR dry matter. The value observed in present experiment was both lower and higher than calculated value of metabolizable energy of TMR. The metabolizable energy of TMR (3.137±0.056 Mcal kg^{-1} DM) significantly improved at 5.0% MOM incorporation in comparison to control TMR (1.685±0.047 Mcal kg^{-1} DM). Further higher level of MOM incorporation was without significant effect on improvement of energy levels. An incorporation of 5% and higher level was found sufficient to improve energy content of TMR.

Nouala et al. (2006) reported that the ME content of *Moringa oleifera* forage harvested at 30, 40 and 50 days post pruning was higher (10.97, 10.98 and 9.92 MJ kg^{-1} DM, respectively; $P<0.01$) in comparison to second cut alfalfa hay (8.33 MJ kg^{-1} DM) at 24 hour in vitro incubation utilizing ewe rumen fluid. In present study, ME content of TMRs with *Moringa oleifera* meal was higher than reported by Nouala et al. (2006) owing to TMR has 50% concentrate ingredients and concentrate component was replace with 2.5 to 22.5 *Moringa oleifera* meal on protein equivalent basis. The incorporation of *Moringa oleifera* meal 5% and above up to 22.5% in TMR to replace high protein concentrate mixture was found suitable to improve ME content of rations.

An *in vitro* investigation (Lunagariya et al., 2017) of exogenous fibrolytic enzymes (EFE) @ 0, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380 and 400 mg kg^{-1} TMR in the ration of dairy cows weighing 500 kg and producing 12 kg 4% fat corrected milk (FCM) per day, resulted in the significantly ($p<0.05$) higher and optimum in vitro digestibility of DM (63.03%) and OM (63.62%) as well as Total Gas Production (72.35 ml 500 mg^{-1} TMR) at supplementation of 240 mg EFE kg^{-1} TMR, along with better ME (7.16 MJ kg^{-1} DM) and MBP (97.63 mg 500 mg^{-1} TMR) content of ration. An *in vivo* investigation on optimum EFE (240 mg kg^{-1} TMR) in crossbred cows yielded significantly higher 4% FCM and milk fat with improved feed conversion efficiency and better economic returns in HF crossbred cows (Lunagariya et al., 2019a) and improved digestibility of dry matter and nutrients as well as higher body weight gain in dry non-pregnant Gir and crossbred dairy cows (Lunagariya et al., 2019b). An application of *in vitro* investigation to reduce numbers of treatments and animals for *in vivo* investigation was found beneficial along with economically effective, less time consuming and allows more control over experiment as reported by Makkar (2004). Looking to these investigations and present study, it can be suggested that an incorporation of *Moringa oleifera* meal @ 5.0 with increment levels of 2.5% and higher levels up to 22.5% to replace high protein concentrate mixture in total mixed ration found suitable for *in vivo* investigation.

4. CONCLUSION

An incorporation of *Moringa oleifera* meal @ 5.0 with increment levels of 2.5% up to 22.5% to replaced high protein concentrate mixture (50%) in TMR improved ($p<0.05$) dry matter digestibility (60.879±0.251 to 64.678±0.679%), gas production (81.000±9.018 to 95.667±1.764 ml 500 mg^{-1} TMR) and ME content (2.356±0.068 to 3.223±0.057 Mcal ME kg^{-1} TMR) of total mixed ration which was suitable for *in vivo* growth experiment.

5. ACKNOWLEDGEMENT

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