



Evaluation of Total Mixed Ration with *Moringa Oleifera*

Sharma et al.

Physio-Clinical Evaluation of Total Mixed Ration with *Moringa Oleifera* Leaves For Crossbred Calves

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ABSTRACT

The experiment was planned to evaluate the effect of 50% protein base incorporation of *Moringa oleifera* leaves in TMR on physio-clinical parameters in crossbred calves. The calves with uniform body weight were allocated sequentially to control (T1) and *Moringa oleifera* leaves MOL (T2) groups, having four female and three male calves in each. The calves in T1 and T2 groups were fed TMR prepared using calf starters and calf starters of which 50% crude protein was replaced with *M. oleifera* leaves. The calves were fed only calf starters during the first week and then transferred to TMR of the respective group. The effect on the physiological response, haematology, serum biochemistry, faecal condition score, and faecal microbial content was evaluated. Feeding MOL T2 TMR to crossbred calves were having a non-significant effect on physiological parameters (rectal temperature, pulse rate, respiration rate), blood haematology, serum energy protein nutrition metabolites (glucose, total protein, albumin, globulin), liver function metabolites (AST, ALT), and kidney function metabolites (creatinine, BUN) with a beneficial effect on gut health. The physio-clinically 50% incorporation of MOL in TMR of crossbred calves was safe on physiological parameters, blood haematology, and serum metabolites with beneficial effects on gut health.

KEYWORDS: Crossbred calves, Gut health, Haematology, *Moringa oleifera* leaves, Physiological parameters, Serum metabolites

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INTRODUCTION

Feed deficiency and the high rearing cost of ruminants are the challenges for dairy farmers in developing countries. To economize dairy production in developing countries, cheap and easily available unconventional feedstuffs are imperative (Oduro et al., 2008). *Moringa (Moringa oleifera)* leaves are a potential protein source (Soltan et al., 2012) with an additional source of bioactive compounds. *Moringa* leaves protein is considered an alternative to concentrate protein source (Soliva et al., 2005; Shershya et al., 2022) for dairy animal diets. The leaves have 20-26% crude protein (CP) and higher minerals and vitamins (Soltan et al., 2017; FAO, 2014). *Moringa oleifera* leaves and other parts contain various phytochemicals with high potency as anti-fertility, cardiogenic, anti-cancerous, anti-anthelmintic, anti-tubercular, anti-spasmodic,

abortifacient, anti-inflammatory and anti-microbial properties (Sholapur and Patil., 2013; Wang et al., 2016). These phytochemicals are saponins, terpenoids, tannins, quercetin, kaempferol, sterols, anthraquinones, glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate (Berkovich et al., 2013; Shah et al., 2016; Saini et al., 2016; El Desoky et al., 2017). The plant leaves protein also contains various anti-nutritional factors like protein digestion and utilization inhibitor substance, energy utilization limiting substances, increasing vitamin requirement and immune antagonist substances. *Moringa oleifera* leaves contain alkaloids, flavonoids, oxalates, phytate, saponin, and tannin @ 1.56, 5.42, 1.42, 2.33, 2.06 and 1.63% (Stevens et al., 2015) which affect the growth performance. The phenolic compound like tannins can be complex with trypsin and amylase reducing protein and energy availability. The saponins

in leaves can be responsible for the bitter taste and reduce feed intake. The oxalate can be complex with calcium reducing availability. These necessitate the physio-clinical evaluation of *Moringa oleifera* leaves (MOL) in the crossbred calves. With this idea in mind, the experiment was planned to evaluate the effect of 50% protein base incorporation of MOL in TMR on physio-clinical parameters in crossbred calves.

MATERIAL AND METHODS

Animals and dietary treatment

The present experiment was conducted at Livestock Research Station (LRS), Anand Agricultural University, Anandas per directives of Institutional Animal Ethics Committee. The farm is located 43 meters above sea level with 20° 35' N latitude and 72° 55' longitude. The experiment was conducted for 126 days from July 2022 to February 2023 using fourteen crossbred calves (75% Holstein Friesian x 25% Kankrej). The calves with uniform body weight were allocated sequentially to control (T1) and *Moringa oleifera* leaves (MOL; T2) groups, having four female and three male calves in each. The calves in T1 and T2 groups were fed TMR prepared using calf starters and calf starters with 50% crude protein replaced with *M.oleifera*

leaves (Table 1). The calves were fed only calf starters during the first week and then transferred to TMR of the respective group. The leaves of PKM-1 variety of *Moringa oleifera* (MOL) were used to prepare calf starters of T2 group after drying under a shed and crude protein analysis (AOAC, 1995). The new-borns calves were weaned at birth, and first fed colostrum within one hour of birth, the remaining quantity was adjusted in the second feeding. The colostrum was fed during the first three days and then shifted to milk. The calves were fed 12, 16, 12, 8, and 4% milk of body weight in 1, 2-4, 5-7, 8-10, and 11-13 weeks of age, respectively two times in equal half at Morning and Evening.

The experimental calves were housed in East-West oriented dome-shaped shed with an individual feeding facility. The shed was cleaned daily with water and disinfected with phenyl to maintain hygienic conditions. Every day, calves were let loose for two hours in the morning under controlled conditions for exercise, during this period they have access to clean and fresh water. The water was also offered two times at the tying place. The calves were dewormed using fenbendazole/piperazine on the 15th and 30th days of age and subsequently at 30 days intervals.

Table 1. The ingredient proportion of TMRs (% on dry matter basis) fed to crossbred calves

Ingredients proportion (kg)	T1	T2	T1	T2
Concentrate to Roughage ratio	75:25		65:35	
Period of feeding (weeks)	2-13		14-18	
Calf starter inc. 0.5 kg premix*	75.0	37.5	65.0	32.5
<i>Moringa oleifera</i> leaves-MOL	0.00	37.5	0.00	32.5
Sorghum Hay	15.0	15.0	17.2	17.2
Green Hybrid Napier	10.0	10.0	17.7	17.7
Total	100.0	100.00	100.00	100.00

* Premix specification Calcium: 202 g; Phosphorus: 118 g; Magnesium: 10 g; Sodium: 5.4 g; Sulphur: 9 g; Zinc: 12.76 g; Copper: 1 g; Cobalt: 0.125 g; Manganese: 4 g; Selenium: 0.03 g; Iodine: 0.4 g; Iron: 6 g; Chromium: 0.02 g; Vitamin A: 1100000 IU; Vitamin D3: 220000 IU; Vitamin E: 200 mg; Vitamin B12: 8.8 mg; Niacin: 2759 mg; 5000 mg and 10000 mg Methionine activity and Lysin per kg, respectively.

Physiology and blood evaluation

Rectal temperature ($^{\circ}\text{F}$), respiration rate (breaths/min.), and heart rate (beats/min.) were recorded before feeding and watering at biweekly intervals. The blood from each crossbred calf was collected at the initial (0 days), mid (63 days), and final (126 days) of the experiment. The blood was collected aseptically from the jugular vein of calves in Ethylene Diamine Tetra-acetate (EDTA) and clot activator Vacuette. The whole blood collected in the EDTA Vacuette was used to analyse the haematological profile employing Mindray BC 2800 VET cell counter machine. The serum was separated from the clot activator Vacuette using REMI research centrifuge machine at 550g for 10 minutes and stored at -20°C for the biochemical profile of the calves employing CKK 300 Automatic Biochemistry Analyser. The Glucose (mg/dL) was estimated by GOD-POD method, Calcium (mg/dl) by Arsenazo III method, Serum Aspartate Aminotransferase (AST)/ Serum Glutamic Oxaloacetic Transaminase (SGOT) (U/L) and Serum Alanine Aminotransferase (ALT)/ Serum Glutamic Pyruvic Transaminase (SGPT) (U/L) by Modified IFCC method, serum creatinine (mg/dL) by Modified Jaffe's method, Blood Urea Nitrogen (BUN) (mg/dL) by GLDH-UREASE method, Total protein (g/dL) by Biuret method, and albumin (g/dL) by BCG Dye method while the Globulin (g/dL) and Albumin: Globulin ratio were calculated, using the analytic values of total protein and albumin.

Faecal evaluation

The faecal consistency scores were given from 1 to 4, modified from 0 to 3 as described by Renaud et al. (2020). Score 1 suggests normal consistency, score 2 suggests soft and does not hold firm, score 3 suggests runny and spreads readily, and 4 indicates watery liquid consistency. The faecal sample from the rectum of four calves from each treatment was collected in a sterile container during the last week of the experiment. The samples were stored at 4°C for analysis. The specific diluted sample was inoculated on an agar count plate for the total viable bacterial and viable coliform count. The inoculated Petri plates were incubated at 37°C for 72 hrs in the incubator for the growth of bacterial colonies. The total viable bacterial count in the faeces was determined as the standard plate count method as described by Quinn et al. (1994). The intestinal protozoa-Coccidia was estimated four times at the interval of a week after 95 days of the experiment from faeces of four calves from each treatment and termed as Coccidia oocyst per gram (OPG), employing the Stoll's dilution technique (Pal & Sanyal, 2020).

Statistical Analysis

The data were presented as mean \pm SE and were analyzed as per standard methods (Snedecor and Cochran, 2014) via one-way ANOVA by means of Web Agriculture Statistic Package- WASP 2.0 developed by Jangam and Wadekar, (2004). At <0.05 probability, the difference between the treatment means was considered significant.

RESULTS AND DISCUSSION

Physiological response

Table 2. Physiological responses of crossbred calves fed MOL in TMR

Parameter	T1	T2	P value
Rectal temperature ($^{\circ}\text{F}$)	102.4 \pm 0.05	102.3 \pm 0.13	0.69
Pulse rate (beats/min.)	96.7 \pm 1.14	101.0 \pm 0.94	0.10
Respiration rate (breaths/min.)	35.4 \pm 1.02	37.6 \pm 1.27	0.03

Where MOL=*Moringa oleifera* leaves; TMR=Total mixed ration

The physiological parameters viz., rectal temperature and the pulse rate were showing a non-significant difference between the two groups while the respiration rate in the T2 was significantly higher than that of T1. All physiological parameters' values were within the physiological range (Silva et al., 2016; Piccione et al., 2010), indicating that the inclusion of 50% protein base MOL in TMR replacing calf starter was safe to feed to crossbred calves. Similarly, Wafa et al. (2017) reported a non-significant influence on the rectal temperature and pulse rate of buffalo bulls fed 4% *Moringa oleifera* leaves whereas respiration rate was significantly higher, which contrasts the findings of this study.

The fluctuating and lower rectal temperature in the initial periods (Figure 1) in this experiment, might

be due to the incompletely developed hypothalamus at birth, and also various intrinsic and extrinsic factors (Silva et al., 2016). The higher pulse rate of crossbred experimental calves at initial life in this experiment, might be due to lower systolic volume and the requirement of higher blood quantity at the organ level, forcing the heart to beat at high frequency (Piccione et al. 2010). The respiration rate of calves was higher in the initial period at birth and the initial age which become normal during subsequent age. Silva et al. (2016) have mentioned that 14 days are required to fully functionalize the lungs and carry out the integrated gas exchange. As in neonatal life, the anatomical and functional capacity of the lungs is not complete.

Table 3. Haematological parameters of crossbred calves fed MOL in TMR

Parameter	T1	T2	P value
WBC (*10 ³ /μl)	9.31±0.41	9.61±0.30	0.69
LYM (*10 ³ /μl)	4.74±0.16	4.53±0.50	0.51
MON (*10 ³ /μl)	0.67±0.10	0.68±0.09	0.47
NEU (*10 ³ /μl)	4.62±0.45	4.56±0.36	0.20
EOS (*10 ³ /μl)	0.05±0.00	0.08±0.04	0.25
BAS (*10 ³ /μl)	0.01±0.00	0.01±0.00	0.87
RBC (*10 ⁶ /μl)	7.43±0.37	8.22±0.49	0.79
Hb (g/dl)	9.97±0.40	8.88±0.13	0.07
HCT (%)	26.0±0.79	25.9±0.50	0.60
MCV (fl)	33.4±0.47	35.1±0.50	0.48
MCH (pg)	9.51±0.27	10.4±0.43	0.87
MCHC (g/dl)	29.3 ^b ±1.72	31.2 ^a ±0.34	0.01

Where MOL= *Moringa oleifera* leaves; TMR= Total mixed ration; WBC=White Blood Cells; LYM= Lymphocytes; MON=Monocytes; NEU= Neutrophils; EOS= Eosinophils; BAS= Basophils; RBC= Red Blood Cells; Hb=Haemoglobin; HCT=Haematocrit; MCV=Mean Corpuscular Volume; MCH= Mean Corpuscular Haemoglobin; MCHC= Mean Corpuscular Haemoglobin Concentration; μl= Microlitre, dl= Decilitre; fl= Femtolitre; pg= Picogram.

Physiology and blood evaluation

The values of white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils, red blood cells, haemoglobin, haematocrit value, mean corpuscular volume and mean corpuscular haemoglobin were significantly not influenced by 50% MOL in TMR of crossbred calves whereas mean corpuscular haemoglobin concentration was significantly higher in T2 group of calves. The values of the above-mentioned parameters were within the normal physiological range (Mohri et al., 2007; Kaneko, 2008; Radostitis et al., 1994). This indicated

that the incorporation of 50% protein base MOL in TMR replacing calf starters was without adverse effect and can be safely included in the ration of crossbred calves. The findings of Sherasiya et al. (2023) on the haematological values like WBCs, RBCs, Hb, haematocrit, and platelet count on 5 and 7.5 per cent incorporation of MOM in the TMR of crossbred heifers, supporting our research statement. While higher values of lymphocytes, neutrophils, eosinophils, and haemoglobin were observed by Mandal et al. (2015) in fluorosis-affected calves fed 25g *Moringa oleifera* fruit powder.

Table 4. Serum metabolites of crossbred calves fed MOL in TMR

Parameter	T1	T2	P value
Glucose (mg/dl)	101.3±3.18	99.5±4.23	0.13
Total protein (g/dl)	6.66±0.11	6.71±0.17	0.13
Albumin (g/dl)	3.33±0.08	3.19±0.11	0.98
Globulins (g/dl)	3.34±0.15	3.53±0.23	0.21
A:G ratio	1.05±0.08	0.98±0.09	0.60
SGPT/ALT (U/L)	27.8±0.32	27.8±0.19	0.69
SGOT/AST (U/L)	67.6±4.84	75.2±4.90	0.47
Creatinine (mg/dl)	1.14±0.03	1.31±0.07	0.47
BUN (mg/dl)	20.6±0.67	21.17±0.90	0.77
Ca (mg/dl)	10.8±0.29	10.9±0.10	0.22

Where MOL= *Moringa oleifera* leaves; TMR= Total mixed ration; A: G= Albumin to globulin ratio; SGPT= Serum Glutamic Pyruvic Transaminase; ALT= Serum Alanine Aminotransferase; SGOT= Serum Glutamic Oxaloacetic Transaminase; AST= Serum Aspartate Aminotransferase; BUN= Blood Urea Nitrogen; Ca= Calcium

The energy and protein nutrition metabolites (serum glucose, total protein, albumin, globulins, and albumin to globulins ratio) concentration of crossbred calves were statistically similar in both groups and were within the physiological range (Hussein et al., 2020). A similar finding on serum glucose concentration was observed in crossbred heifers (Sherasiya et al., 2023) fed 5 and 7.5% *Moringa* meal, Holstein dairy calves (Zeng et al., 2017) fed *M. oleifera* silage and Pantaneira calves (Chiodi et al., 2014) fed *Moringa* hay. While a higher

concentration of serum glucose (Kakena et al. 2020; Khalel et al. 2014) in lactating cows was reported on feeding *Moringa* containing TMR. The serum total protein, albumin, and globulin level observed by Parmar et al. (2021) in Gir calves, Sherasiya et al. (2023) in crossbred heifers, and Aharwal et al. (2019) in Murrah buffalo calves on the feeding of *Moringa* were similar to the present findings. While Khalel et al. (2014) reported a higher total protein value on the feeding of *Moringa*.

The liver function serum metabolites like serum glutamic pyruvic transaminase/serum alanine aminotransferase (ALT), and serum glutamic oxaloacetic transaminase/ serum aspartate aminotransferase (AST) of crossbred calves in both groups were statistically similar. In accordance with the present findings, Parmar et al. (2021), and Sherasiya et al., (2023)denoted a non-significant effect over serum ALT and AST levels on feeding *Moringa* in the diet of Gir calves, and crossbred heifers, respectively. Contrarily, Elaidy et al. (2017) observed lower serum AST values in the *Moringa*-fed group of buffalo calves.

The kidney function metabolites like creatinine and blood urea nitrogen (BUN) concentration in both groups of crossbred calves were statistically similar. The report of Sherasiya et al. (2023), Aharwal et al.

(2019), Elaidy et al. (2017), and Chiodi et al. (2014) on creatinine and blood urea nitrogen was as per the findings of the present study, while higher BUN level was observed by Zeng et al. (2017) in Holstein dairy calves. The serum calcium level also followed the same trend, indicating sufficient calcium was available from *M. oleifera* leaves.

All these serum parameters on energy protein nutrition, liver and kidney function of crossbred calves were within the physiological range as reported by Hussein et al. (2020) and Pandya et al. (2022). The serum metabolites concentration indicated that 50% MOL can be incorporated in TMR replacing 50% protein base replacement of calf starter.

Faecal evaluation

Table 5. Faecal condition score of crossbred calves fed MOL in TMR

Parameter	T1	T2	P value
Average daily score	1.24 ^a ±0.03	1.08 ^b ±0.02	0.000
Frequency distribution of daily FCS			
Score 1 (Normal)	105.2±2.76 (83.56 %)	117.8±1.42 (93.54%)	
Score 2 (Soft)	12.7±2.17 (10.09 %)	5.43±1.04 (4.31 %)	
Score 3 (Very soft)	6.43±0.87 (5.10 %)	1.14 ±0.34 (0.91 %)	
Score 4 (Liquid watery)	1.71±0.29 (1.36 %)	1.00±0.38 (0.79 %)	

Where MOL= *Moringa oleifera* leaves; TMR= Total mixed ration

The daily faecal consistency score was significantly lower in the T2 group, which was indicative of more days of normal faecal consistency compared to the control T1 group. The frequency of normal FCS (Score 1) was higher and the abnormal score was lower in 50 per cent MOL T2 group compared to the control group. This FCS were indicative of *M. oleifera* leaves usefulness to maintain normal gut health of calves in T2 TMR group.

Diarrhoea (Gakuya et al., 2014), dehydration, pathogen infection causing bacteraemia and sepsis (Aharwalet al., 2018) are important health problems found in dairy calves. The reduction in days of diarrhoea, and more normal faecal consistency is beneficial to the health of calves and production economy. The findings of Kekana et al. (2021) in Holstein calves and Aharwal et al. (2019) in buffalo calves fed *Moringa oleifera* leaf supports the present findings.

Table 6. Microbial evaluation of faeces of crossbred calves fed MOL in TMR

Parameter (log ₁₀ value)	T1	T2	P value
Total viable bacterial count (cfu)	9.16±0.85	10.5±0.09	0.15
Coliform count (cfu)	6.02±0.26	5.97±0.19	0.87
Coccidial oocyst (OPG)	3.25 ^a ±0.01	2.53 ^b ±0.06	0.00

Where MOL= Moringa oleifera leaves; TMR= Total mixed ration; cfu = Colony forming unit; OPG= Oocyst per gram.

The colony-forming unit of Total viable bacteria was numerically increased and Coliform bacteria were numerically reduced on feeding MOL in T2 TMR. The coccidial oocysts in faeces were significantly lower in the T2 group than in the control T1 group. The numerically higher total viable bacteria, numerically lower coliform bacteria and significantly lower coccidial oocyst in T2 group are indicative that MOL incorporation in TMR is beneficial to the gut health of crossbred calves.

El-Moez et al. (2014) reported reduced faecal heterophilic count in rabbits fed 0.15 and 0.30% *M. oleifera* dried leaves diet, which supports the present findings. Alimirzaei et al. (2019) reported $7.7 \times 10^6 - 1.1 \times 10^8$ (log₁₀ 6.89-8.04) coliform count in faeces of Holstein female calves fed milk @ 20% of body weight and Bayatkouhsar et al. (2013) also reported coliform as a 2.490-2.570 (log₂) colony forming unit (cfu) in faeces of normal-health Holstein female calves. This supports our findings on coliform cfu per gram of faeces in crossbred calves. The faecal coccidial oocyst count (OPG) in a normal healthy goat was 4.60-4.79 logarithmic values as reported by Ravindra Kumar et al. (2022). The reduction in faeces coccidial oocyst was reported by Olounladé et al. (2021) in naturally infected rabbits, AbdelTawab et al. (2020) in infected Albino mice and El Banna et al. (2016) in non-infected broiler chicken fed *ad-lib*. *M. oleifera* leaves, 400 mg Moringa leaf extract per kg body weight, and *M. oleifera* (0.5 and 1%), respectively supports the present findings.

CONCLUSION

The feeding of *Moringa oleifera* leaves in TMR replacing 50% calf starter to crossbred calves was safe on physiological parameters (rectal

temperature, pulse rate, respiration rate), blood haematology, serum energy protein nutrition metabolites (glucose, total protein, albumin, globulin), liver function metabolites (AST, ALT), and kidney function metabolites (creatinine, BUN) with beneficial effect on gut health.

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