

Assessment of *Moringa oleifera* meal supplementation on the antioxidant status of crossbred heifer calves

Asif N. Sherasiya¹, Pravin M. Lunagariya^{2*}, Jigar H. Patel², Mohsinkhan M. Pathan³, Mahesh M. Chaudhary² and Malhar R. Khant²

¹Animal Nutrition Research Station, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat

²Livestock Research Station, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat

³Dept. of Veterinary Biochemistry & Physiology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat

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ABSTRACT

The experiment was conducted to evaluate the anti-oxidant effect of *Moringa oleifera* meal-supplementation in the total mixed ration, replacing the compounded concentrate mixture for crossbred heifer calves. A total of twenty-one crossbred heifer calves were selected and grouped into three treatments based on body weight and age. The leaves (84%) and soft twigs (16%) of *Moringa oleifera* were mixed to prepare the *Moringa oleifera* meal. Compounded concentrate mixture, ground nut straw, hybrid napier grass, mineral mixture and common salt were used at 50, 23, 25, 1 and 1%, respectively to formulate the control total mixed ration (T1). The compounded concentrate mixture was replaced with the *Moringa oleifera* meal at 5.0 and 7.5 % to formulate total mixed rations T2 and T3. The whole blood was collected from each calves in the morning before feeding and watering at the beginning (0 day), middle (at 60 day) and end (at 126 day) of the experiment. The total leukocyte counts, total erythrocyte counts, haematocrit and platelet count of crossbred heifer calves differed non-significantly ($P>0.05$) between the treatment groups, and were within normal physiological range. The serum albumin, glucose, creatinine, urea, alkaline phosphatase, alanine amino transaminase, calcium and phosphorus differed non-significantly between the treatment groups. However, feeding with *Moringa oleifera* resulted in higher ($P<0.01$) serum total protein (T2 and T3) and also higher ($P<0.05$) aspartate aminotransferase values (T2). The activity of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and thiobarbituric acid reactive substances, were also non-significantly ($P>0.05$) higher after feeding with the 5.0 and 7.5% *Moringa oleifera* meals. Feeding with 5.0 and 7.5% *Moringa oleifera* meal in the total mixed ration, replacing the high protein compounded concentrate mixture, to growing crossbred heifer calves had no adverse effect on haematological and biochemical constituents, and resulted in a non-significant ($P>0.05$) higher antioxidant capacity in the crossbred heifer calves.

Key words: anti-oxidant; crossbred heifer calves; haematology; liver and kidney function; *Moringa oleifera* meal

*Corresponding author:

Lunagariya, Pravin M., Assistant research scientist, Livestock Research Station, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, e-mail: drpravinml@gmail.com

Introduction

Moringa (*Moringa oleifera*) is a popular multipurpose legume, from a fast-growing, and deciduous tree that usually grows to 10-12 m in height (MISHRA et al., 2012). *Moringa* leaves are abundant in protein, amino acids, fatty acids, minerals, vitamins, calcium, potassium, and various phenolics and oxycarotenoids which are the basic building blocks and can improve the antioxidant capacity of the animal body (DESHMUKH, 2014). Given the high biological value of *Moringa* leaves, they have the potential for use as feed for ruminants (PRADHAN, 2016). Many parts of the *Moringa* tree are recommended for use as supplementation when minerals and vitamins are limited or unavailable. In addition, *M. oleifera* leaves and other parts contain various phytochemicals with high potency, such as: cardiogenic, anti-cancerous, anthelmintic, anti-tubercular, anti-spasmodic, abortifacient, anti-inflammatory and antimicrobial properties (SHOLAPUR and PATIL, 2013; WANG et al., 2016). These phytochemicals include 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). STEVENS et al. (2015) quantified the alkaloids, flavonoids, oxalates, phytate, saponin and tannin from *Moringa oleifera* leaves as 1.56, 5.42, 1.42, 2.33, 2.06 and 1.63%, respectively, and they all affect animal performance. The phenolic compounds, such as tannins, can be complex, with trypsin and amylase reducing protein and energy availability. The saponins in the leaves may be responsible for the bitter taste and reduce feed intake. The phytates and oxalate content of *M. oleifera* leaves is complex, with phosphorus and calcium along with microminerals. The oxalate content is lower than that of spinach and green amaranth leaves (RADEK and SAVAGE, 2008).

In the light of these facts, the experiment was planned to evaluate the effect of *Moringa oleifera* meal supplementation in the total mixed ration, replacing a compounded concentrate mixture, on antioxidant activity in crossbred dairy heifer calves.

Materials and methods

The study was conducted for 126 days (nine bi-week period; 9*14 days) at the Livestock Research Station, Anand Agricultural University, Anand, Gujarat, India. Crossbred heifer calves were cared for and managed as per the directives of the Institutional Animal Ethics Committee (permit no. 322/LRS/2020). In total, twenty-one crossbred heifer calves (75% Holstein Friesian x 25% Kankrej) of similar body weight (110.1±5.1, 111.8±7.6 and 109.8±4.8 kg for T1, T2 and T3) and age (184±22, 178±24 and 177±24 days for T1, T2 and T3) were selected and grouped in three treatment groups with seven calves in each. The PKM-1 variety of *Moringa oleifera* was shown for study. The first pruned at 75 days after showing and subsequently at 60 day intervals, leaving a 1.5 feet stem from the ground for regrowth. The harvested *Moringa* fodder was sun dried for 3 days in a cemented yard. The leaves and soft twigs were mixed at 84% and 16%, respectively, to prepare a *Moringa oleifera* meal (MOM). The soft twigs were ground in a hammer mill using a 2mm sieve to mix with the leaves. Compounded concentrate mixture (CCM), groundnut straw (GNS), hybrid napier grass (HN), mineral mixture (MM) and common salt were used at 50, 23, 25, 1 and 1%, respectively, to formulate the control total mixed ration (T1). The compounded concentrate mixture was replaced with MOM at 5.0 and 7.5% to formulate the TMR for groups T2 and T3. The mineral mixture contained 200g calcium, 120g phosphorus, 50g magnesium, 18-30g sulphur, 6.25g zinc, 1g copper, 125mg cobalt, 4g manganese, 30mg selenium, 400mg iodine, 20mg chromium, 5 million IU vitamin A, 1million IU vitamin D3 and 150mg vitamin E per kilogram weight. Ground nut straw was ground and hybrid napier grass was chaffed to 5mm to incorporate into the TMR. Fresh TMR was prepared daily on a clean cement floor and fed to the crossbred heifer calves as per the NRC (2001) standards to meet their nutrient requirements.

The crossbred heifer calves were tied individually in a well-ventilated barn for feeding and care. They were fed individually according to their treatment group, twice (morning-10:00 hrs

and afternoon-15:00 hrs). The crossbred calves were set loose for two hours in the morning under controlled conditions. Fresh, clean and wholesome drinking water was available during these two hours and also offered where they were tied at 14:00 and 18:00 hrs. The calves were dewormed before and after three months of the experiment with a broad spectrum anthelmintic. The TMRs and ingredients were analysed for proximate and fibre fractions as per AOAC (1995) and VAN SOEST et al. (1991), respectively.

Blood samples were collected from each crossbred calf in the morning before feeding and watering (8:00 to 9:00 hrs) on day 0, the 60th day and the 126th day of the experiment. Blood samples were collected from the jugular vein using a Vacuette taking all aseptic precautions in two separate Vacuettes: EDTA and a clot activator. Blood collected in the EDTA Vacuette was analysed after completion of blood collection for haematological parameters using a Mindray BC-2800Vet haematology analyser. Serum was separated from the clot activator Vacuettes using a REMI research centrifuge machine after centrifuging at 550g for 10 minutes. Serum was collected in a sterilized Eppendorf tube and stored

at -20°C for further analysis. The biochemical parameters were analysed using coral clinical system kits (Tulip group, India) in a Mindray BS-120 automatic chemistry analyser. The antioxidant enzymes superoxide dismutase, glutathione peroxidase and thiobarbituric acid reactive substances were assessed using Cayman ELISA kits (Cayman Pharma, Czech Republic). The mean of all three samplings was use for analysis of variance. Experimental data were presented as means and standard deviation, and the mean values were analysed using SPSS software as per SNEDECOR and COCHRAN (1991). Significant differences between the means of different treatments were assessed by Least Square Difference, and the differences between the treatments were declared significant at $P < 0.05$.

Results

The mean values of the chemical composition and fibre fractions of the ingredients and TMRs are presented in Table 1. The proximate and fibre fraction composition of all the TMRs were similar. The *Moringa oleifera* meal contained 24.97% crude protein on a dry matter basis.

Table 1. Chemical composition of feeds, fodder and TMR on % DM basis

Ingredient	CCM	HN	GNS	MOM	T1	T2	T3
OM	92.66	89.52	86.03	86.66	86.27	86.59	87.45
CP	25.48	7.60	10.29	24.97	18.80	18.49	18.61
EE	4.27	2.44	2.12	4.04	3.66	3.75	3.61
CF	16.57	34.43	33.04	12.29	19.57	19.22	19.32
NDF	39.80	68.08	60.68	28.78	53.22	53.18	53.47
ADF	28.32	52.87	52.88	21.52	36.41	36.89	37.31
NFE	46.34	45.05	40.58	45.36	44.24	45.13	45.91
Ash	7.34	10.48	13.97	13.34	13.73	13.41	12.55

TMR=Total Mixed Ration, DM=Dry Matter, CCM= Compound Concentrate Mixture, HN= Hybrid Napier, GNS= Ground Nut Straw and MOM= *Moringa oleifera* Meal, OM=Organic Matter, CP=Crude Protein, EE=Ether Extract, CF=Crude Fibre, NDF=Neutral Detergent Fibre, ADF=Acid Detergent Fibre, NFE=Nitrogen Free Extract

The results for haematological parameters (Table 2) such as the total leukocyte counts, total erythrocyte counts, haemoglobin, haematocrit and platelet counts of the crossbred heifer calves differed non-significantly ($P>0.05$) between the treatment groups, and were within the normal physiological range.

The biochemical parameters are presented in Table 3. The level of serum total protein was significantly ($P<0.01$) higher in ascending order

from T1 control to T3, and without any effect of treatments on serum albumin and glucose level. The mean aspartate amino transferase (AST) concentration was significantly higher ($P<0.05$) in T2 (86.77 ± 11.88) as compared to T1 (75.10 ± 8.29) and T3 (82.45 ± 12.92), while the serum values of creatinine, urea, alkaline phosphatase, alanine amino transferase (ALT), calcium and phosphorus differed non-significantly between the treatment groups.

Table 2. Haematological parameters of crossbred heifer calves

Parameter	T1	T2	T3	P value
WBC ($10^3/\mu\text{l}$)	9.17 \pm 1.88	9.23 \pm 2.27	10.35 \pm 2.28	0.280
RBC ($10^6/\mu\text{l}$)	8.48 \pm 1.11	9.02 \pm 1.25	8.69 \pm 1.22	0.484
Haemoglobin-Hb (g/dl)	10.15 \pm 1.19	10.11 \pm 1.76	9.42 \pm 1.36	0.339
Haematocrit-HCT (%)	33.20 \pm 4.73	33.89 \pm 6.49	31.87 \pm 4.86	0.608
Platelet ($10^3/\mu\text{l}$)	496.35 \pm 111.86	472.14 \pm 105.98	509.42 \pm 115.03	0.669

WBC=White blood cell, RBC=Red blood cell

Table 3. Serum parameters of crossbred heifer calves

Parameter	T1	T2	T3	P value
Protein and Energy metabolism				
Total protein (g/dl)	5.49 ^a \pm 0.16	6.24 ^b \pm 0.28	6.44 ^a \pm 0.27	<0.01
Albumin (g/dl)	3.33 \pm 0.52	3.23 \pm 0.51	3.27 \pm 0.48	0.868
Glucose (mg/dl)	80.84 \pm 5.27	79.73 \pm 5.31	79.44 \pm 6.65	0.797
Kidney and Liver function				
Creatinine (mg/dl)	1.15 \pm 0.20	1.18 \pm 0.23	1.06 \pm 0.22	0.332
Urea (mg/dl)	8.27 \pm 0.22	8.27 \pm 0.09	8.27 \pm 0.17	0.989
Alkaline phosphatase (U/L)	160.35 \pm 25.12	159.84 \pm 30.90	161.97 \pm 29.29	0.979
SGOT/AST (U/L)	75.10 ^b \pm 8.29	86.77 ^a \pm 11.88	82.45 ^b \pm 12.92	<0.05
SGPT/ALT (U/L)	37.36 \pm 4.09	37.64 \pm 3.28	38.35 \pm 3.63	0.773
Major minerals				
Calcium (mg/dl)	9.46 \pm 0.32	9.47 \pm 0.33	9.47 \pm 0.35	0.995
Phosphorus (mg/dl)	6.53 \pm 0.47	6.54 \pm 0.43	6.54 \pm 0.39	1.000

SGOT=Serum glutamic oxaloacetic transaminase, AST=Aspartate aminotransferase, SGPT=Serum glutamic pyruvic transaminase, ALT=Alanine aminotransferase

Means with different superscripts (a, b and c) in a row differ significantly ($P<0.05$)

The results for the serum antioxidant enzymes are presented in Table 4. The differences in concentrations of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and thiobarbituric acid reactive substances

(TBARS) were non-significant ($P>0.05$) between the treatments. The non-significantly higher antioxidant enzyme activity was suggestive of the improved antioxidant capacity of crossbred heifer calves after feeding 5.0 and 7.5% MOM containing TMR.

Table 4. Serum anti-oxidant capacity of crossbred heifer calves

Parameter	T1	T2	T3	P value
SOD (U/ml)	0.0121±0.00	0.0128±0.00	0.0131±0.00	0.581
GPX (nmol/min.)	6091.24 ±256.67	6131.80 ±528.77	6284.98 ±529.38	0.502
TBARS (µM)	9.56±0.95	9.75±1.40	9.62±1.08	0.910

Note: SOD= Superoxide dismutase, GPX=Glutathione peroxidase, TBARS=Thiobarbituric acid reactive substance

Discussion

The *Moringa oleifera* meal contained 24.97% crude protein on a dry matter basis and contained leaves and soft twigs at 84 and 16 per cent, respectively. XIE et al. (2020) and DEY et al. (2014) reported a slightly higher and comparable leaf crude protein (27.6 and 26.34±0.67%, respectively) content of *Moringa oleifera*. However, SERADJA et al. (2019) reported lower crude protein (20.1-21.0% of DM) in *Moringa* forage-leaves, twigs and new buds harvested at 30, 40 and 50 days after pruning. NOUALA et al. (2006) also reported slightly lower crude protein (23.27%), neutral detergent fibre (18.74%) and acid detergent fibre levels (16.07%) of *Moringa oleifera* leaves. This variation may be due to the different agro-climatic zones, soil types, different maturity stage, and also may be due to variety of *Moringa*. All three TMRs contained similar approximate nutrients and fibre fractions.

All haematological parameters, such as total leukocyte counts, total erythrocyte counts, haemoglobin, haematocrit and platelet counts, were within the normal range and also in accordance with the normal range reported by AGGARWAL et al. (2016) and JONES and ALLISON (2007) in cattle. Similar results were also reported by SONAKAR et al. (2020) after feeding 10 and 20% *Moringa oleifera* leaves in a commercial compounded

concentrate mixture to lactating Sahiwal cows. The findings of this experiment suggest that there is no harmful or detrimental effect on the haematological values from feeding TMR with 5.0 and 7.5% MOM.

In the present experiment, we found significantly ($P<0.01$) increased levels of serum total protein in groups T2 and T3 as compared to the control T1. This might be due to the good supply of limiting amino acids in MOM (HENUK, 2018). However, the values also remained within the physiological range (AIELLO et al., 2016). The feeding of rumen protected the limiting amino acids lysine and methionine (MOVALIYA et al., 2013), and in free or protected forms (MAZINANI et al., 2020) resulted in improved serum protein through efficient nitrogen use in growing animals. The level of serum aspartate amino transferase also increased significantly ($P<0.01$) as compared to the control group after feeding *Moringa oleifera* meal, which indicates improved liver function. However, the value was within the normal range (AGGARWAL et al., 2016). All other serum parameters also remained within physiological range, in accordance with AIELLO et al., 2016; AGGARWAL et al., 2016; KANEKO et al., 2008; ABD ELLAH et al., 2014. This indicates that 5.0 and 7.5% *Moringa oleifera* meal can be used as a supplement in the rations of crossbred heifer calves without any detrimental effect on liver and kidney function.

The serum levels of albumin, globulin, glucose, triglycerides, cholesterol, high density lipoprotein, low density lipoprotein and creatinine differed non-significantly ($P>0.05$) after feeding a control diet and a diet with 15 kg *Moringa* green fodder, replacing green hybrid napier grass, in crossbred cows (SHANKHPAL et al., 2019). The feeding of *Moringa oleifera* silage replacing 25% alfalfa hay + 50% maize silage and 50% alfalfa hay + 100% maize silage in the diet of Holstein dairy cows had non-significant ($p>0.05$) effects on the serum ALT, glucose, albumin and triglyceride concentrations (ZENG et al., 2018). A significantly ($P<0.05$) higher serum AST value was reported by ELAIDY et al. (2017) after feeding a 5% dry *Moringa oleifera* leaf (DMOL) ration, compared to 10, 15 and 20% DMOL rations, in suckling buffalo calves. However, KEKANA et al. (2019) reported significantly higher ($P<0.05$) serum albumin, glucose and IgG levels, and significantly lower ($P<0.05$) creatinine after micro-supplementation of a *Moringa oleifera* leaf meal in lactating Jersey cows. The discrepancies between studies on the effect of *Moringa oleifera* might be due to differences in quantities and states (dry, silage, green) of inclusion in the diet as well as the proportion of diet replaced.

The improvement in the antioxidant capacity of crossbred heifer calves after feeding 5.0 and 7.5% MOM in TMR was statistically non-significant. However, KEKANA et al. (2019), KHALEL et al. (2014) and SHANKHPAL et al. (2019) reported significantly improved antioxidant capacity after feeding a *Moringa oleifera* containing diet to lactating Jersey, Friesian crossbred cows, respectively. The difference in antioxidant response might be due to differences in quantities and states (dry, silage and green) of *Moringa oleifera* usage as the sun drying of leaves reduces ascorbic acid, polyphenol and flavonoid content (LUQMAN et al., 2012; SANTOS et al., 2012).

Conclusions

Feeding *Moringa oleifera* meal in a total mixed ration, replacing a high protein compounded concentrate mixture, in growing crossbred heifer calves had no adverse effect on haematological

parameters such as white blood cell, red blood cell, haemoglobin, haematocrit and platelet counts. The serum parameters, such as albumin and glucose, creatinine, urea, alkaline phosphatase, and alanine amino-transaminase were also not adversely affected. However, feeding *Moringa oleifera* resulted in higher ($P<0.01$) serum total protein (T2 and T3) and ($P<0.05$) aspartate aminotransferase values (T2). The effect on antioxidant capacity was also non-significant ($P>0.05$).

Conflict of Interest

The authors declare that there is no conflict of interest for this research study.

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SHERASIYA, A. N., P. M. LUNAGARIYA, J. H. PATEL, M. M. PATHAN, M. M. CHAUDHARY, M. R. KHANT: Učinci konjske rotkvice (*Moringa oleifera*) kao dodatka prehrani na antioksidacijski status junica križanki različitih pasmina. Vet. arhiv 93, 399-406 2023.

SAŽETAK

Ovo je istraživanje provedeno kako bi se procijenio antioksidacijski učinak konjske rotkvice (*Moringa oleifera*) kao zamjene za mješavinu koncentrata u hranidbi junica križanki različitih pasmina. Na temelju tjelesne mase i dobi, junice (n=21) su razvrstane u tri pokusne skupine. Lišće (84%) i mekše grančice (16%) konjske rotkvice izmiješane su kako bi se pripremio obrok za istraživane životinje. U formulaciji mješavine (T1) upotrijebljena je mješavina koncentrata u količini od 50%, mljeveni orašasti plodovi 23%, *Hybrid Napier* 25%, mineralna mješavina 1 % i obična sol 1 %. Pripremljena smjesa koncentrata zamijenjena je obrokom s konjskom rotkvicom u omjeru 5,0% i 7,5% za skupine T2 i T3. Od svake je junice uzet uzorak pune krvi ujutro prije hranjenja i pojenja na početku (0. dan), sredinom (60. dan) i na kraju pokusa (126. dan). Nije bilo statistički znakovite razlike ($P > 0,05$) u ukupnom broju leukocita i eritrocita, hematokritu te broju trombocita među pokusnim skupinama i vrijednosti su bile unutar fiziološkog raspona. Ni u vrijednostima serumskog albumina, glukoze, kreatinina, ureje, alkalne fosfataze, alanin-aminotransaminaze, kalcija i fosfora nije bilo statistički znakovite razlike među pokusnim skupinama. Dodatak prehrani konjske rotkvice međutim rezultirao je porastom proteina u serumu ($P < 0,01$) (skupine T2 i T3) i porastom vrijednosti alanin-aminotransferaze ($P < 0,05$) (u skupini T2). Ni aktivnost antioksidacijskih enzima poput superoksid-dismutaze, glutation-peroksidaze i reaktivne tvari tiobarbiturinske kiseline nije bila znakovito veća ($P > 0,05$) pri hranjenju obrocima s dodatkom konjske rotkvice. Prehrana s 5,0 i 7,5% dodatka konjske rotkvice koja je zamijenila visokoproteinsku koncentriranu mješavinu u obrocima junica nije utjecala na njihove hematološke i biokemijske pokazatelja kao ni na njihov antioksidacijski kapacitet čije neznatno povećanje nije bilo statistički znakovito ($P > 0,05$).

Ključne riječi: antioksidant; junice križanci; hematologija; bubrežna i jetrena funkcija; obrok s dodatkom *Moringa oleifera*
