Moringa oleifera leaf meal as a feed supplement for dairy calves

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Abstract

The study evaluated the effects of supplements of *Moringa oleifera* leaf meal (MOLM) on blood biochemistry and growth of pre-weaned Holstein calves (n = 48). Four-day-old calves were blocked (based on order of birth and birth weight) and randomly allocated to three dietary treatments, namely M0: unsupplemented control, M30: supplemented with 8.33 g MOLM /100 kg bodyweight (BW), and M60: supplemented with 16.66 g MOLM /100 kg BW. The experiment ended when the calves were 42 days old. The MOLM was diluted in the milk of the morning feeding session. Feed intake, faecal score, and body temperature were recorded daily. Bodyweight and body measurements were taken weekly, and blood samples were collected at the same time. Starter dry matter intake (DMI) for the M60 group was higher than for M0 group with a significant week effect. Calves in M60 had higher final bodyweight and body measurements than M0 and M30. Similar trends were observed for superoxide dismutase (SOD), catalase (CAT), and serum antioxidant activity (SAOA). Immunoglobulin G (Ig) levels increased with the age of calves in both M30 and M60 groups, with the increase being progressively larger with the level of MOLM supplementation. Calves in the M60 group had fewer days of diarrhoea. Supplementation of MOLM supplied secondary metabolites and nutrients that improved feed utilization and later stimulated growth, increased enzymatic antioxidants, and reduced diarrhoeal incidence in pre-weaned calves. Dilution of MOLM by as little as 16.66 g/100 kg BW in the milk for dairy calves can be recommended.

Introduction

Management of replacement heifers is a crucial component of dairy production (Hawkins *et al*., 2019). Neonatal calves do not produce enough gamma globulin, and the onset of breathing generates reactive oxygen species (ROS), which can result in oxidative stress (Wiedemann *et al*., 2003). Feeding high-quality colostrum is critical in conferring passive immunity to new-born calves (Besser & Gay, 1994; Godden, 2008; Stelwagen *et al*., 2009). However, colostrum has few antioxidants and is rich in pro-oxidants (Kankofer & Lipko-Przybylska, 2008; Sordillo, 2016) that induce oxidative stress (Albera & Kankofe, 2011). Failure to transfer passive immunity also contributes to oxidative stress and poor growth performance in early life (Ling *et al*., 2018). Severe oxidative stress is associated with diarrhoea and pneumonia in the first 21 days of life (Ranjan *et al*., 2006). Neonatal diarrhoea is as a major disease of calves and contributes to the risk of calf mortality (Windeyer *et al*., 2014). Interventions to counteract birth-associated oxidative stress by increasing their pool of antioxidants are critical for calf growth.

Several researchers (Khalel *et al*., 2014; Kholif *et al*., 2016; Falowo *et al*., 2018; Kekana *et al*., 2019, 2020) suggested synthetic anti-oxidants as alternatives to minimize the effects of ROS with different levels of success. From an organic perspective, *Moringa oleifera* has attracted attention because of the high concentrations of antioxidant, antimicrobial, and immunomodulatory properties inferred by flavonoids (Kholif *et al*., 2016; Dong *et al*., 2018; Falowo *et al*., 2018). Moreover, the leaves of MO are rich in minerals (Ca, K, Zn, Mg, Fe, and Cu) and vitamins (A, B, C, D, and E) (Mbikay, 2012) and are preferentially browsed by many species of birds, possibly because of these attributes (Nherera-Chokuda, 2021, personal observation). The potential of MO leaves as immunity boosters in mature cows and goats has been noted (Kekana *et al*., 2019; Kekana *et al*., 2020; Kholif *et al*., 2016). However, its effects on the maintenance of oxidative stress, energy balance, health, and growth performance in calves have not been fully investigated. Hence, this study evaluated the effects of pre-weaning supplementation of MOLM on immunity, diarrhoea control, and growth performance in dairy calves.

Material and methods

This experiment was conducted at the Agricultural Research Council - Animal Production Institute at Irene, South Africa (28013' 0" S; 25055' 0" E, altitude 1523 m) during the November 2017 to January 2018 calving season. Ethical clearance was granted by the Institute's ethical review committee (APIEC 17/008).

After calving, cows were monitored closely, and suckling was prevented. After cleaning, calves were separated from the dams and taken to the calf unit where birth weight was determined. Colostrum samples were collected and tested with a colostrometer (Biogenics, Napa, Calif, USA) for quality control and fed to calves within six hours of birth. Then the calves were fed colostrum-rich milk twice daily (09h00 and 14h00) at the rate of 10% of their birth BW until day 3. The calves were housed individually in 15 m^2 pens consisting of concrete and dirt ground (concrete floor with a rubber mat and *Eragrostis curvula* hay for cold shock. The concrete floor (8 m^2) had a roof, and the remaining area was dirt exercise. Pens were cleaned every other day with a pressured hose and disinfectant. Only 18 of 30 pens were used twice, so were cleaned and disinfected (Formalin, ANB Vet, Midrand) and left dry for seven days before housing the new calf. Throughout the trial, health was observed daily.

Forty-eight new-born female Holstein calves (32.5 ± 4.5 kg BW) were used in this study. The calves were randomly allocated at birth to one of three treatment groups. Month of birth was considered a block, and treatments were allocated within the block. Treatments were based on levels of MOLM, namely M0: control, M30: 8.33 g MOLM/100 kg BW and M60: 16.66 g MOLM/100 kg BW. A portion of milk (100 ml) from the calf's daily allowance was warmed to 40 °C and mixed with the amounts of MOLM for each treatment. The mixture was returned to the milk feeding bucket and fed to each calf from day 4 to 42 days old. The composition of MOLM was as described in Kekana *et al*. (2020) and the levels were adjusted weekly to maintain 8.33/100 kg and 16.66/100 kg BW.

The milk feeding regime is shown in Table 1. Between day 4 and day 42, calves received starter feed (Table 2) and fresh clean water at libitum. Calf starter, milk and water intakes were recorded daily as the difference between amounts offered every morning and refusals, collected the following morning before the next feeding.

Table 1 Colostrum and milk feeding regime of calves supplemented with *Moringa oleofera* leaf meal

Feedstuff	Days	09:00	14:00
Colostrum[1]	0 - 3	10% BW	10% BW
Milk[2]	4 - 7	2L	2L
Milk	8 - 14	3L	3L
Milk	15 - 35	2L	2L
Milk	36 - 42	2L	-

[1]Dry matter: 23.2%, crude protein: 15.9%, fat: 5.23%, lactose: 3.42%, immunoglobulin G: 58.3 g/L, total antioxidant capacity: 2962.2 mmol Trolox-equivalent/g protein
[2]Crude protein: 3.23%, fat: 3.47%, lactose: 4.69%

Composite daily milk samples from the dairy were collected weekly in the morning and evening and pooled to determine their CP, fat and lactose content using a System 4000 infrared analyser (Foss Electric, Hillerød, Denmark). Calf starter was sampled, and oven dried at 90 °C for 24 hours to determine its DM content. Ground samples were analysed for CP according to method 968.06, and ether extract (EE) according to method 920.39 (AOAC, 2000). Calcium, potassium, and phosphorus were assayed according to method 965.17(AOAC, 2000). Neutral detergent fibre was determined following Van Soest *et al*. (1991).

The calves were weighed at birth and at seven-day intervals until weaned at 42 days old. Bodyweight was measured using a digital weighing scale (TAL-TEC®, Brits, South Africa) and bodyweight change was calculated. Heart girth was defined as the circumference of the thoracic cavity immediately behind the forelimbs, and body length was the distance from point of the shoulder to the point of *tuber ischia*. All measurements were taken in the morning before feeding.

Table 2 Chemical composition of starter ration[1] fed to calves received *Moringa oleofera* leaf meal as feed supplement during pre-weaning period

Nutrient	Quantity provided
Dry matter, g/kg	88.2
Crude protein, g/kg DM	182
Fat, g/kg	35.0
Neutral detergent fibre, g/kg	218
Metabolizable energy (calculated), MJ/kg of DM[2]	14.3
Calcium, g/kg	8.0
Phosphorus, g/kg	5.0

[1] Premix provided vitamin A: 17000 IU, vitamin D: 3000 IU, vitamin E: 30 mg, niacin: 125 mg, manganese: 50 mg, Iron: 50 mg, zinc: 50 mg, copper: 10 mg, iodine: 0.8 mg, selenium: 0.15 mg, phosphorus: 180 mg, antioxidant: 50 mg

Blood samples were collected before feeding and treatment allocation on day 3, and before feeding on days 12, 22, 32, and 42. Samples were collected from the jugular vein using a 10-ml vacutainer tube (ANB Vet, Centurion, South Africa). Then the samples were kept at room temperature for a minimum of 1 hour to allow clotting to occur before centrifugation at 1764 × g for 15 minutes. Serum was decanted into three aliquots and stored at -20 °C until analysis.

Beta-hydroxybutyrate (βHB) (Williamson *et al.*, 1962), non-esterified fatty acids (NEFA) (De Villiers *et al.*, 1977) and glucose (Bradford, 1976) were analysed. The activities of superoxide dismutase (SOD) (Beauchamp & Fridovich, 1971), glutathione peroxidase activity (GSH-Px) (Paglia & Valentine, 1967), catalase activity (CAT) and malondialdehyde (MDA) (Tug *et al.*, 2005) were also assayed, and serum antioxidant activity (SAOA) was assayed according to Koracevic *et al.* (2001).

Immunoglobulin G (IgG) concentration was analysed with an ELISA kit for bovine IgG from Bio-X Diagnostics (Jemelle, Belgium). All kit components were brought to 21 °C before use. The wash buffer was diluted twentyfold with distilled water. A calibration curve was developed and samples were diluted at 1/1000 in PBS according to the manufacturer's instructions. Diluted samples were added to the test plate in duplicate and incubated at 21 °C for 1 hour. The test plate was then washed three times with the wash buffer before a chromogen solution (100 µL) was added to each well and incubated away from light for approximately 10 minutes. Finally, stop solution (50 µL) was added to each well and the optical densities were recorded with a microplate spectrophotometer with a 450-nm filter. An interassay CV of <0.15 was observed. The concentration of IgG in samples was calculated from the standard reference curve provided in the test kit.

Body temperature was measured daily between 8h00 and 9h00 with a rectal thermometer (Dt-K111B, ANB Vet, Midrand, South Africa). The procedure was repeated twice for accuracy. All occurrences and treatments of lung diseases and diarrhoea were noted individually. Lung infection was defined as coughing or sneezing for two days or more or heavy breathing with additional signs such as nasal discharge. Diarrhoea was defined as soft watery faeces lasting for two days or more, or as soft or watery faeces in combination with impaired condition and possible weight loss.

Faecal scoring was conducted daily (08h00) to determine faecal fluidity and consistency on a scale of 1–4, in which 1 = formed stools, 2 = soft or moderate consistency, 3 = start of the diarrhoea (mild) or runny, and 4 = watery faeces or profuse diarrhoea (Larson *et al.*, 1977). Number of days of diarrhea (duration), diarrheal occurrence (frequency) and severity (faecal score) were recorded.

The MIXED procedure of SAS version 9.0 was used to analyse the data (SAS Institute Inc., Cary, North Carolina, USA). The linear model included fixed effects of block, treatment, time, and two- and three-way interactions, with the identity of the calf included as a random effect. No block, block × treatment, block × time, and block × treatment × time effects were identified. Time was used as a repeated measure with a first-order autoregressive covariance structure, which provided the best fit according to Schwartz's Bayesian information criterion. Degrees of freedom were calculated using the Kenward-Roger option, and the means were determined with the least-squares means statement. Effects were considered significant at $P < 0.05$ and existence of a trend was accepted when $0.05 < P < 0.10$.

Results and Discussion

Intake of solid and liquid feeds increased with the level of MOLM. Both levels of MOLM increased starter DMI compared with the control group (*P* =0.01). Mean milk and water intakes for the M60 group were higher than M0 (*P* =0.01). Feed and water intake was 28% and 14% higher in M60 compared with M0 and M30, respectively. However, there were no differences between M60 and M30. A similar trend was noted on final bodyweight (*P* =0.04). Average daily gain tended to be higher in M60 (0.48 kg/day) (*P* =0.06) relative to control (0.39 kg/ day). When evaluated weekly, weight in the M60 group increased (*P* =0.02) and was higher in week 4 (Figure 1). The body measurements of heart girth and body length increased significantly (*P* <0.01) with MOLM levels. Means for feed intake and growth performance are presented in Table 3.

Table 3 Intake and growth performance of neonatal Holstein calves as affected supplementation with *Moringa oleofera* leaf meal during the pre-weaning period

Trait	Treatments			SE	*P*-value		
	M0 (n = 16)	M30 (n = 16)	M60 (n = 16)		Treatment	Week	Treatment x Week
Starter DMI, kg/d	0.35[c]	0.42[b]	0.49[a]	0.01	0.01	<0.01	0.16
Milk intake, L/d	3.25[b]	3.76[ab]	4.40[a]	0.21	0.04	0.01	0.27
Total DMI, kg/d	0.68[b]	0.81[ab]	0.94[a]	0.01	0.01	<0.01	0.21
Water intake, L/d	2.00[b]	2.28[ab]	2.92[a]	0.55	0.04	0.02	0.22
Initial BW, kg	32.4	32.6	32.5	1.24	0.52		
Final BW, kg	54.2[b]	57.5[ab]	62.8[a]	1.49	0.04		
Average gain, kg/d	0.40	0.43	0.48	0.02	0.06	0.01	0.11
Body length, cm	70.3[b]	75.9[ab]	80.2[a]	5.63	0.02	<0.01	0.24
Heart girth, cm	85.1[b]	88.4[ab]	92.1[a]	3.85	0.04	<0.01	0.35

DMI: dry matter intake; BW: body weight; M0: unsupplemented control, M30: *Moringa oleifera leaf* meal supplemented at 8.33 g MOLM /100 kg BW/day, M60: *Moringa oleifera leaf* meal supplemented at 16.66 g MOLM/100 BW/day
[a,b,c] Within a row, means with a common superscript did not differ with probability *P* =0.05
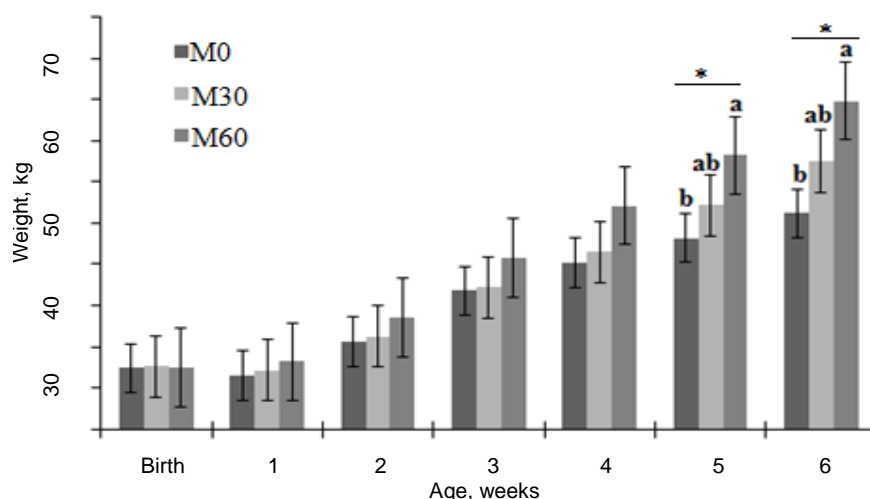

Figure 1 Weight change of neonatal Holstein calves (n = 16) supplemented with *Moringa oleofera* leaf meal

M0: unsupplemented control, M30: supplemented with 8.33 g *Moringa oleifera* leaf meal /100 kg BW/day, M60: supplemented with 16.66 g *Moringa oleifera* leaf meal /100 kg BW/day; * 0.01 < *P* < 0.05
[a,b] Within a week, bars headed by a common letter indicate means that were not different at *P* =0.05

Mean antioxidant status (SOD, CAT, GSH-Px, and SAOA), energy metabolites (βHBA, glucose and NEFA) and immunity (IgG) are presented in Table 4. With the exception of MDA, the activities of oxidative

enzymes increased with the level of MOLM. The activity of SOD from M60 was the highest (P <0.01) relative to M0 and M30 calves, with M0 being the least. A similar trend was observed with M60 having the highest CAT (P <0.001) and SAOA (P <0.01). SAOA increased from 12 days old (P <0.01) until the end of the trial (Figure 2). The concentration of MDA was reduced by the inclusion of MOLM (P <0.001). The activity of GSH-Px also increased with the incremental levels of MOLM (P =0.03), with more significant effect over time (week).

Table 4 Blood metabolites and antioxidant enzymes of neonatal Holstein calves as affected by supplementation with *Moringa oleofera* leaf meal during pre-weaned period

Status	Treatment			SE	P-value		
	M0 (n = 16)	M30 (n = 16)	M60 (n = 16)		MOLM	Day	MOLM x day
Antioxidant							
SOD, u/mg protein	3.46[c]	6.32[b]	10.21[a]	0.24	<0.01	<0.01	<0.01
GSH-Px, ug/mg protein	12.3[b]	14.5[b]	17.2[a]	0.47	0.03	<0.01	0.12
CAT, u/mg protein	3.86[c]	6.52[b]	10.31[a]	0.62	<0.01	<0.01	<0.01
MDA, mol/L	0.86[a]	0.62[b]	0.38[c]	0.02	<0.01	<0.01	<0.01
SAOA, µmol/L	32.1[c]	49.8[b]	65.5[a]	2.27	<0.01	<0.01	<0.01
Energy							
βHBA, mmol/L	0.09[b]	0.15[ab]	0.18[a]	0.05	<0.01	<0.01	0.02
Glucose, mmol/L	5.34[b]	5.73[b]	7.28[a]	0.19	0.01	0.02	0.14
NEFA, mmol/L	0.19[a]	0.16[b]	0.11[c]	0.24	0.02	<0.01	0.04
Immunity							
IgG, g/L	10.9[b]	13.8[ab]	15.9[a]	0.25	0.02	0.04	0.05

IgG: immunoglobulin G, βHBA: beta-hydroxyl-butyric acid, NEFA: non-esterified fatty acids, SOD: superoxide dismutase, GSH-Px: glutathione peroxidase, MDA: malondialdehyde, CAT: catalase, SAOA: serum total antioxidant capacity, M0: unsupplemented control, M30: *Moringa oleifera leaf* meal supplemented at 8.33 g MOLM /100 kg BW/day, M60: *Moringa oleifera leaf* meal supplemented at 16.66 g MOLM/100 BW/day
[a,b,c] Within a row, means with a common superscript did not differ with probability P =0.05
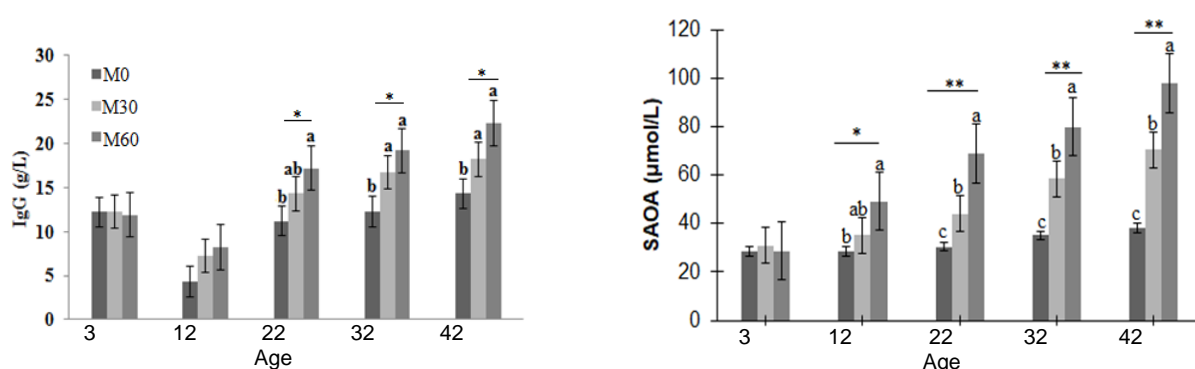

Figure 2 Immunoglobulin G and serum antioxidant activity levels of neonatal Holstein calves (n = 16) supplemented with *Moringa oleifera* leaf meal

M0: unsupplemented control, M30: supplemented with 8.33 g *Moringa oleifera* leaf meal /100 kg BW/day, M60: supplemented with 16.66 g *l*eaf meal /100 kg BW/day
IgG: immunoglobulin G; SAOA: serum antioxidant activity levels
* 0.01 < P < 0.05, ** P < 0.01

Calves fed M60 had higher (*P* <0.01) βHBA than M0, but M30 did not differ from the M0 group. Similarly, glucose levels did not vary between M0 and M30, but both groups had lower concentrations (*P* =0.01) than the M60 calves. Non-esterified fatty acid concentrations were lowest (*P* <0.01) in M60 (0.11 mmol/L) compared with M30 (0.17 mmol/L) and M0 (0.19 mmol/L) with a significant week effect (*P* <0.01), and treatment-week interaction (*P* =0.04) effect. The IgG levels were higher in M60 calves (*P* =0.02) than M0, but M60 and M30 did not differ (*P* >0.05). When evaluated over time, IgG for the MOLM groups increased with age (*P* =0.04) with a significant increase being noted from day 22 for M60 (Figure 2).

The M60 calves had the fewest days of diarrhoea (*P* <0.01) and lowest diarrhoea frequency (*P* =0.04), with a noticeable week effect (*P* <0.01, *P* = 0.01) (Table 5). *Moringa oleifera* leaf meal
tended (*P* =0.08) to reduce the faecal score. Calves in the M0 group received electrolytes for more days (4 days) compared with the M60 group (2 days), and only 3 days in M30. The effects of MOML were not observed on lung disease and body temperature.

Table 5 Pre-weaning diarrhoeal incidence and health status of Holstein calves as affected by supplementation with *Moringa oleifera* leaf meal

Measurement	Treatments			SE	*P*-value		
	M0 (n = 16)	M30 (n = 16)	M60 (n = 16)		MOLM	Week	MOLM x Week
Days of diarrhoea	4.82[a]	3.25[b]	2.06[c]	0.06	<0.01	<0.01	0.06
Diarrheal occurrence	1.78[a]	1.52[ab]	1.34[b]	0.23	0.04	<0.01	0.02
Faecal score	2.83	2.25	2.06	0.02	0.08	<0.01	0.15
Body temperature, °C	37.3	38.0	38.0	6.59	0.23	<0.01	0.37
Occurrence of lung disease	0.32	0.21	0.20	0.04	0.14	<0.01	0.18

M0: unsupplemented control, M30: *Moringa oleifera leaf* meal (MOLM) supplemented at 8.33 g MOLM /100 kg BW/day, M60: *Moringa oleifera leaf* meal supplemented at 16.66 g MOLM/100 BW/day
[a,b,c] Within a row, means with a common superscript did not differ with probability *P* =0.05

Moringa oleifera and its by-products have been investigated extensively in ruminant nutrition because of its relatively high biomass yield, high nutritional quality, and low cost of production. Hence, in this study, antioxidant enzymes, energy metabolites, health, and growth performance were investigated when pre-weaned Holstein calves were fed increasing levels of MOLM.

The current data showed that milk intake rose with the increasing levels of MOLM, though an unexpected reduction in milk intake was noted from control calves, which could have been from loss of appetite as a result of sickness (diarrhoea) (Heath *et al.*, 1987). The increased milk in MOLM-supplemented calves could be attributed to the changed taste and the aroma of the milk, which stimulated appetite. In this study, the dilution of MOLM in milk may have acted as a sialagogue and stimulated the secretion of saliva, which made swallowing easier. Herbal supplements enhance and add flavours to animal feed, and therefore influence eating patterns, secretion of digestive fluids and total feed intake (Bhatt, 2015).

Milk and water intakes were stimulated in MOLM groups, and also intake of starter feed. Stimulated feed intake in neonatal calves is associated with higher nutrient digestibility, promoted reticular rumen weight, papillae width and papillae density as a result of elevated ruminal butyric acid (Muya *et al.*, 2017). In Kholif *et al.* (2016), a 25% increase in ruminal butyric acid concurrent with enhanced nutrient digestibility was observed in Nubian goats that were supplemented with MOLM. It could therefore be inferred that MOLM supplementation induced ruminal butyric acid production, which accelerated rumen development and stimulated nutrient digestibility in calves, although ruminal butyric acid was not measured in the current study. This resulted in increased feed intake providing a greater quantity of energy that was reflected in increased energy metabolites (glucose and βHBA). Higher serum glucose level is essential in calves as it serves as the primary source of energy before rumen development. However, as consumption of solid feed increases (beyond 21 days) and the rumen begins to develop, the contribution of volatile fatty acids (VFAs) to the calves' energy increases, resulting in a higher level of βHBA (Muya *et al.*, 2015). This could explain the elevated βHBA levels in calves treated with MOLM.

There is a dearth of literature about the effects of flavonoids and phenolic-rich herbs on the performance of pre-weaned dairy calves. However, Frutos *et al.* (2004) reported that rumen microorganisms in calves at 21 days old could tolerate low and moderate concentrations of secondary metabolites, including

phenolic compounds and tannins, without negative effects on rumen fermentation. Phenolic compounds and tannins inhibit ruminal methanogenesis in the rumen environment of the matured ruminants, resulting in efficient repartition of the energy consumed in methane and milk production (Aerts *et al.*, 1999). Supplementation of MOLM to mature goats resulted in increased in VFA and propionate concentrations in the rumen (Kholif *et al.*, 2014), the key substrates for gluconeogenesis. Collectively, these characteristics explain the greater performance in this study (Figure 1). Elaidy *et al.* (2019) observed increased weight gain and feed/gain ratio from MOLM supplementation in buffalo calves. Ahmed and Shaarawy (2019) reported a significant growth performance in final weight gain, total weight gain, average daily gain and feed conversion efficiency in goat kids that received *Moringa* leaves.

The current data showed that MOLM could reduce the blood NEFAs and the activity of MDA. In calves, NEFA levels are established maternally through colostrum feeding (Ling *at al.*, 2018) and may directly induce cellular oxidative stress (Inoguchi *et al.*, 2000). The high levels of NEFA alter gene expression that is related to lipid and carbohydrate metabolism in bovine embryos (Van Hoeck *et al.*, 2015). Malondialdehyde is another degradation product of lipid peroxidation formed under oxidative stress conditions (Nielsen *et al.* 1997). Hence, the reduced levels of malondialdehyde reflect i) reduced risk of oxidative stress, ii) reduced lipid peroxidation and iii) pronounced energy balance in dairy cattle (Caré *et al.*, 2018; Ling *et al.*, 2018). In this study, NEFA levels were much lower than those reported by Ling *et al.* (2018). Similarly, reduced NEFA levels after MOLM supplementation were observed in mature cows (Kekana *et al.*, 2019; Shankhpal *et al.*, 2019; Kekana *et al.*, 2020), mature goats (Kholif *et al.*, 2016; Ahmed & Shaarawy, 2019), attributable to the increased feed intake that provided greater quantities of energy. Similarly, Kirisattayakul *et al.* (2013) reported significantly attenuated MDA levels with *M. oleifera* leaf extract at a dose of 100 mg.kg^{-1} BW in rats. In Serafini *et al.* (2011), *M. citrifolia* rich with flavonoids acted against lipid peroxidation and hydroxyl and nitric-oxide-induced radicals and improved antioxidant activity. Although low NEFA levels and reduced MDA activity are desirable, they also play a role in curbing production of cellular free radicals (ROS) indirectly by stimulating inflammatory pathways under severe lipid peroxidation (Gawel *et al.*, 2004; Ohtsu *et al.*, 2017).

The authors evaluated the activities of SOD, CAT, and GSH-Px as proxy measures for oxidative damage to further determine oxidative stress in calves. During the enzymatic regulation of ROS, SOD is the first enzyme that is involved in the conversion of oxygen radicals to hydrogen peroxide (H_2O_2), whereas CAT is involved in the second step of removing H_2O_2 and converting it to O_2 (Yu, 1994). Also, GSH-Px acts directly on H_2O_2 reduction during free radical mutation (Dröge, 2002). Currently, the activities of SOD, CAT and GSH-Px in serum increased with the level of MOLM and could be attributed to the synergistic antioxidant activities from antioxidant minerals and vitamins (Mbikay, 2012), flavonoids and phenolic (Falowo *et al.*, 2018) in MOLM. Flavonoids (quercetin, kaempferol) were reported to possess abilities to reduce the activity of peroxidase and inhibit the release of free radicals by stimulating the proliferation of neutrophils and the activation of these cells through elevated α1-antitrypsin in vitro (Nijveldt *et al.*, 2001). Moreover, the interaction of MOLM polyphenols with nitric oxide synthases may have inhibited xanthine oxidase (XO), a key source of free radicals. For example, quercetin, silybin and luteolin inhibit the activity of XO, thereby inducing serum activities of antioxidant enzymes (SOD, CAT, and GSH-Px) (Nijveldt *et al.*, 2001). This resulted in balanced redox homeostasis as reflected by the increase in SAOA (Figure 2). Given that SAOA is the determinant of ROS scavenging capability and changes in the redox balance of the living cell (Maciejczyk *et al.*, 2018), the current results highlighted the potentiality of MOLM in curbing the detrimental effects of maternal oxidative stress in neonates during the pre-weaned period. In addition, higher anti-oxidative capacity augments the foundation for sturdy immunity and prevention of subsequent diseases in calves (Nijveldt *et al.*, 2001; Forman, 2016; Sordillo, 2016).

In calves, a solid immune defence that can rapidly inactivate pathogenic invasions is established by adequate serum immunoglobulins (Ig). Therefore, calves with adequate serum IgG will continue to grow normally and not be deterred, as would calves with insufficient IgG (Rodewald, 1976). The current data showed that serum IgG levels increased (Figure 2) with age in all calves. Similarly, Mandal *et al.* (2015) reported that IgG levels increased with sun-dried MOLM supplementation in cattle calves that were exposed to the industrial fluorotic area. In contrast, Elaidy *et al.* (2017) noted low IgG levels, but a significant rise in other immune parameters (leucocytes, plasma fluoride and haematology) of buffalo calves following oven-dried MOLM supplementation. The discrepancy could be because of the differing levels of supplementation, stage of production and breed. In addition, processing methods (extract, oven, sun, and shade dried) affected the levels of the available antioxidants such as ascorbic acids, polyphenols, flavonoids, and organosulfur compounds in MOLM (Aja *et al.*, 2014; Kholif *et al.*, 2016; Falowo *et al.*, 2018), which could have contributed to different physiological responses after supplementation.

The stool fluid content reflects a balance between luminal input (ingestion and secretion of water and electrolytes) and output (absorption) along the gastrointestinal tract (Mukherjee *et al.*, 2011). In the current

study, MOLM calves tended to have a lower faecal score, resulting in a significantly reduced number of days that calves were fed electrolytes. Moreover, the number of diarrhoea days and diarrhoea frequency were significantly reduced in MOLM-fed groups, whereas all calves had the same prevalence of lung disease. This could not be explained so the secondary metabolites responsible for anti-diarrheal activity in MOLM, particularly in ruminants, have yet to be identified. However, the flavonoids in MOLM were reported to exhibit anti-diarrheal activity through inhibition of the intestinal motility and hydro electrolytic secretion in rats (Mukherjee *et al.*, 2011). *Moringa oleifera* flavonoids were also responsible for inhibited hypersecretion and gastrointestinal motility in human beings (Lakshminarayana *et al.*, 2011). Furthermore, the antimicrobial properties in the gut of goats and dairy cows were attributed to the moderate concentrations of phenolic and tannins in *Moringa oleifera* leaf (Kholif *et al.*, 2016; Dong *et al.*, 2018). Reports cited in this study provide evidence of the antidiarrheal activities of flavonoids and phenolic in MOLM and support the authors' observations that *Moringa oleifera* reduced diarrhoeal incidence in supplemented calves.

Conclusion

Moringa oleifera leaf meal supplementation as low as 0.02% BW supplied secondary metabolites and nutrients that improved feed utilization, enzymatic antioxidant status and calf health, reduced oxidative stress, and subsequently stimulated growth. Further studies are needed to determine the optimal intake of MOLM and study its effects on rumen development.

Authors' Contributions

TWK, FVN-C and UM carried out conceptualization, methodology, formal and statistical analysis, investigation, data curation, writing-original draft, editing and visualization, validation, resources, writing review and editing. CMM was involved in project administration, funding acquisition and validation.

Conflict of Interest Declaration

The authors declare there is no conflict of interest and confirm that the manuscript has been read and approved by all authors.

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