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Lactation performance and blood metabolites in lactating dairy cows micro-supplemented with *Moringa oleifera* leaf meal

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Abstract

This study examined the effects of micro-supplementation of *Moringa oleifera* leaf meal on lactation performance and blood metabolites of lactating dairy cows. Thirty Jersey cows (± 40 days in milk (DIM)) were supplemented with *Moringa oleifera* leaf meal (M) at 0 (M0), 30 (M30) and 60 g/cow/day. The experiment lasted until 90 DIM with 14 days adaptation. Milk yield was recorded daily and samples were collected once weekly to determine milk composition and total antioxidant capacity (MTAC). Blood samples were collected on days 54, 68 and 90 in milk to determine serum total protein (TSP), albumin (Alb), immunoglobulin G (IgG) and serum total antioxidant capacity (STAC). Body weight (BW) and milk yield were not affected (P >0.05) by *Moringa* supplementation. *Moringa* supplementation at M60 increased (P <0.05) milk fat and MTAC with a significant reduction in somatic cell count (SCC). Increased (P <0.05) total serum protein (TSP) and IgG with reduced (P <0.05) non-esterified fatty acids (NEFA) levels were observed in M60. Increased (P <0.05) STAC levels were noted in all groups supplemented with *Moringa*. Micro-supplementation with *Moringa oleifera* at 60 g/cow/day markedly reduced oxidative stress, which resulted in improved milk quality and immunity in lactating Jersey cows.

Keywords: antioxidant herbs, antioxidative status, immunity, Jersey cows, milk yield
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Introduction

Dairy farmers are faced with challenges in maintaining production, good health, optimum growth and reproduction, which determine dairy economic feasibility (Barkema *et al*., 2015). Dairy cattle in subtropical areas suffer heat stress, which affects the immune system and ultimately results in mortalities (Kekana *et al*., 2018). Synergistic stress from high temperatures and post-calving negative energy balance (NEB) increase the levels of NEFA (Yatoo *et al*., 2015). High levels of NEFA enhance the oxidation of fatty acids, triggering overproduction of reactive oxygen species (ROS) (Sordillo & Raphael, 2013). The circulation of high and unregulated ROS levels causes oxidative stress, which increases the incidences of inflammatory diseases such as mastitis (Sordillo & Raphael, 2013).

Vitamins, mostly A, C and E, and minerals, such as selenium, copper and zinc, have been used to mitigate oxidative stress (Politis *et al*., 2012). However, in confined systems, the risk of over- or undersupplying micro elements is high (Bouwstra *et al.*, 2010), hence the overproduction of pro-oxides is common (Rizzo *et al*., 2013). Additionally, in semi-intensive feeding systems, trace elements, particularly antioxidant supplementation, are limited owing to restricted or lack of supplementation (De *et al*., 2014). With increasing global food insecurity and malnutrition, especially in developing countries, research focus has shifted to exploration of the potential of natural antioxidants in improving animal production and health to support human nutrition and health.

Moringa oleifera is a natural herbal plant that is recognized for high leaf crude protein (Babiker *et al*., 2017; Babiker, *et al*., 2016) and is utilized in the diets of ruminants and fish. *Moringa oleifera* is native to North India. It is also a rich source of natural antioxidants such as vitamin A (β-carotene), vitamin B (folic

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acid), pyridoxine and nicotinic acid, vitamins C, D, and E (Mbikay, 2012). In addition, *M. oleifera* leaf meal contains phenolic and flavonoids at 31.9 g/100g and 40.8 g/100g, respectively (Pakade *et al*., 2013). These compounds have antioxidative and immunomodulatory properties (Moyo, 2011) that are more effective than synthetic antioxidants such as butylated hydroxytoluene, rutin, and ascorbic acid as noted by Falowo *et al*., (2016). Because early lactating dairy cows are prone to oxidative stress, *M. oleifera* may serve as a natural and cheaper antioxidant source in resource limited-areas. This study therefore evaluated the effects of micro-supplementing *Moringa oleifera* leaf meal on selected blood metabolites and the lactation performance of dairy cows raised in semi-arid conditions.

Materials and methods

The experiment was conducted in Sekhukhune district, Limpopo, South Africa (24.8335 ° S, 29.9741° E). The annual rainfall averages 350 mm with mean maximum temperature of 30 °C and minimum 14 °C.

Thirty second-lactation Jersey cows, weighing 345 ± 40 kg, which were kept under a zero-grazing system, were used in a complete randomized design experiment. At 40 DIM, cows were assigned to one of ten blocks of three cows based on previous lactation performance, Body weight and body condition score. The three treatments consisted of increasing levels of *Moringa oleifera* leaf meal at 0, 30 and 60 g/cow/day, namely M0, M30 and M60. *Moringa* supplementation was top-dressed on the dairy concentrate during morning milking. The animals were fed individually on lucerne hay and *Eragrostis curvula* hay with the commercial lactating concentrate and clean water was supplied ad libitum (Table 1). The concentrate was offered at two equal parts of 3 kg/cow per milking session (9h00 AM and 16h00 PM). The study conformed to the regulations of Agricultural Research Council Ethic Committee (APIEC 14/17) and lasted for 50 days (from 40 to 90 DIM) comprised of 14 days for adaptation and 36 days for sampling.

Table 1 Diet composition and nutrients fed to cows

Diet ingredients (%)	
Concentrates[1]	40.00
Medicago sativa hay	10.00
Eragrostis curvula hay	50.00
Composition	
Dry matter (%)	92.90
Crude protein(%DM)	15.60
Neutral detergent fibre (%DM)	40.80
Acid detergent fibre (%DM)	20.30
Ether extract (%DM)	23.20
Calcium (%DM)	0.32
Phosphorous (%DM)	0.45

%DM: percentage on dry matter basis

[1]The concentrate was purchased from Animal Feed Company, Pretoria, South Africa. Per kilogram the concentrate contained 2 mg/kg sodium chloride, 3.3 mg sodium bicarbonate, 3000 IU vitamin A, 31400 IU vitamin D, 30 IU vitamin E, 100 mg iron, 10 mg copper, 35 mg zinc, 20 mg manganese, 0.3 mg iodine, 0.1 mg selenium, and 0.08 mg cobalt

Moringa leaf meal was purchased from Winterveldt Moringa Farmers' Co-operative in Pretoria, South Africa, and was analysed for phenolic content and antioxidant capacity. The preparation of the plant extract and measurement of total phenolic compounds with Folin-Ciocalteu phenol reagent were conducted using the methods described by Bamdad *et al*. (2006).

To determine the bodyweight change (BWC), the animals were weighed on days 54, 68 and 90 DIM. Daily feed intake was determined as the difference between the amount of feed offered and refusals. Feed samples were composited weekly and ground to pass through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) to analyse dry matter (DM) (AOAC, 2002), neutral detergent fibre (NDF), acid detergent fibre (ADF) (Van Soest *et al*., 1991), crude protein(CP) (Kjeltec 2200, Seattle, WA), ether extracts and minerals (AOAC, 2002).

Cows were hand milked in the morning and afternoon at (09h00 and 16h00). Calves suckled during the day and were separated from their dams overnight. Composite milk samples were collected from

morning milking sessions and analysed for fat, protein, lactose, total solids (TS), solids non-fat (SNF), milk urea nitrogen (MUN) and somatic cell count (SCC) using a MilkoScan analyser (Foss Electric A/S, Hillerød, Denmark). Total antioxidant capacity (MTAC) in milk samples was measured with a modified Trolox equivalent antioxidant capacity (TEAC) method of Van Berg *et al*. (1999).

Blood was collected into the anti-coagulant free vacutainer tubes on 54, 68, and 90 DIM, and centrifuged for 10 minutes at 1000×g. The separated serum was stored in propylene tubes at -20°C pending analysis for total proteins (Ule, 2015), albumin and creatinine (Cr) (Tietz, 1995). Globulin concentrations were calculated as the difference between TP and Alb. Enzymatic methods were used for blood urea determination (BUN) (Tietz, 1995). Immunoglobulins G (IgG) were analysed using the Chemwell auto-analyser and reported as g/L. Analysis of NEFA was done according to Johnson & Peters, (1993) and glucose according to the manufacturer' guide (Glucose HK Gen.3 kit, Lancet Laboratories, Midrand, South Africa). Cholesterol was determined according to Daecon & Dawson (1979). Serum total antioxidant capacity (STAC) was assayed using the method described by Koracevic *et al.* (2001).

Data were analysed using PROC GLM of Statistical Analysis System version 9.0 (SAS, 2009). The three sampling periodic data were regarded as repeated measures with the interaction of treatment and time, as illustrated in the following model:

$$Ycgd = \mu + \alpha g + \beta d + (\alpha\beta)gd + ecgd$$

Where: Ycgd = an observation value for parameters measured from cow c from group g at day of sampling day d
Ycgt = observed variables
μ= overall mean
αg = effect of group g where g = M0 or M30 or M60
βt = effect of sampling day d
(αβ)gt = fixed interaction of effect of group g and sampling day d
ecgt = error associated with the measurement will be taken from cow c, group g at sampling day d

Fisher's least significant difference (LSD) method was used to separate means, difference was declared at $P \leq 0.05$ and tendency at $P < 0.1$.

Results

Total phenolic content (TS) and the 1,1-diphenyl 2-picrylhyorazyl free radical scavenging assay (DPPH-RSA) of *Moringa oleifera* leaf meal were 315.8 mg of TAE/g of DM and 79.5%. Feed intake BW and milk yield were not affected by micro-supplementation (Table 2). This was also observed when evaluated throughout the trial period (Figure 1). Micro-supplementation had no effect on milk quality: milk protein and milk lactose. Milk fat, TS, and non-solid fat were higher ($P < 0.05$) in M60 than in M0 (Table 2). The M60 treatment group had lower SCC than control (2208.7 x 100/ml vs. 2358.5 x 100/m) with no differences ($P > 0.05$) between M30 and M0, and M30 and M60 (Table 3). Increased ($P < 0.05$) MTAC was noted in both M30 and M60 groups, compared with the control. From day 54 of DIM, M60 had higher ($P < 0.05$) MTAC than the rest of the treatments (Figure 2).

Table 2 Milk yield and composition as affected by supplementation of with or without *Moringa oleifera* leaf meal

Treatment	M0	M30	M60	SEM	*P*-value		
					Treat	Day	Treat x day
DMI (kg/d)	12.2	12.9	12.6	0.71	0.489	0.745	0.268
BWC (kg/wk)	1.4	1.4	1.5	0.48	0.61	0.205	0.746
Yield (kg/d)	15.1	15.5	16.8	2.42	0.067	0.122	0.153
Composition of milk							
Fat (g/100g)	3.51^{b}	3.82^{ab}	4.41^{a}	0.45	0.035	0.001	0.082
Protein (g/100g)	3.4	3.5	3.7	0.10	0.066	0.062	0.442
Lactose (g/100g)	4.1	3.81	3.5	0.05	0.063	0.147	0.336
MUN (mg/dl)	10.4^{a}	9.7^{ab}	8.4^{b}	1.07	0.053	0.725	0.188
Total solids (%)	13.1^{b}	13.5^{ab}	14.3^{a}	0.30	<.0001	0.566	0.461
Non-solid fat (%)	7.8^{b}	8.4^{ab}	9.9^{a}	0.50	<.0001	0.576	0.088
SCC x 100/ml	2358.5^{a}	2288.7^{ab}	2208.7^{b}	117.8	<.0001	0.527	0.176
TAC (mmolTE/g protein)	2294.6^{c}	2365.4^{b}	2723.7^{a}	319,4	<.0001	<.0001	0,001

Means with different superscripts ^{abc} differ significantly $P < 0.05$; DMI: dry matter intake, BWC: bodyweight change, MOLM: Moringa oleifera leaf meal, g: gram, mg: milligram, dl: decilitre, MUN: milk urea nitrogen, SCC: somatic cell count, ml: millimetre, TAC: total antioxidant capacity, mmolTE: millimole of trolox equivalent

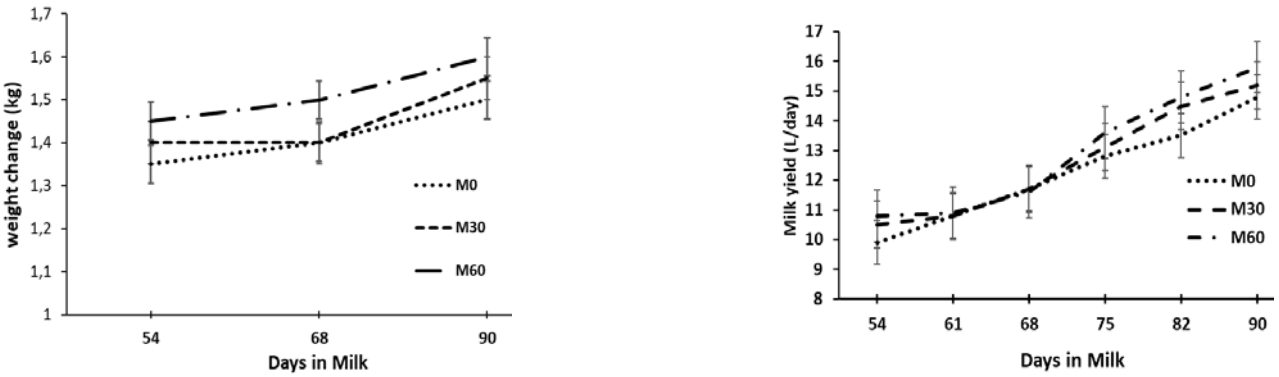

Figure 1 Body weight change and Milk yields in Jersey cows as affected by micro-supplementation of *Moringa oleifera* leaf meal

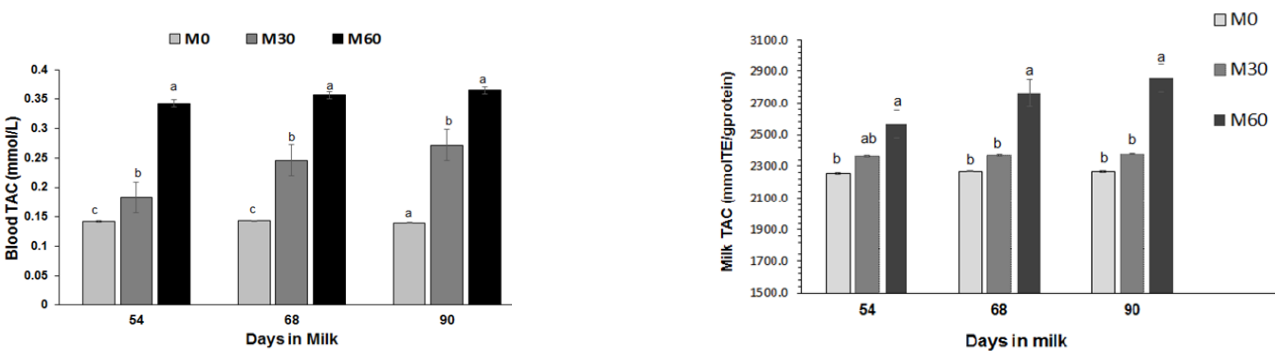

Figure 2 Serum and Milk antioxidant capacities of Jersey cows as affected by the micro-supplementation of *Moringa oleifera* leaf meal. Means with superscripts ^{abc} differ (P <0.05).

Serum proteins, energy metabolites and antioxidant capacity are shown in Table 3 and Figure 2. Cows in M60 had higher TSP, Alb, IgG (Figure 3), and Cre levels than M30 and M0, although differences between M30 and M60 were not significant (Table 3). There were no differences across treatments in total globulin. Cows in the control group had the least blood glucose concentration. Moringa supplementation reduced blood cholesterol levels and increased (P <0.05) STAC (Figure 2) throughout the trial. For the whole study period, non-esterified fatty acids was reduced (P <0.05) in cows supplemented with MOLM (Figure 3).

Table 3 Blood metabolites as affected by supplementation with or without *Moringa oleifera* leaf meal

Treatment	M0	M30	M60	SEM	*P*-Value		
					Treat	Day	Treat x Day
Protein metabolites							
Total protein (g/l)	61.6^{b}	63.1^{b}	68.6^{a}	2.65	0.000	0.085	0.023
Albumin (g/l)	36.1^{b}	36.7^{b}	42.5^{a}	0.65	0.000	0.051	0.215
Total globulin (g/l)	25.5	26.6	26.1	1.5	0.695	0.015	0.223
IgG (g/l)	10.4^{b}	11.8^{ab}	13.6^{a}	0.55	0.000	0.000	0.539
Urea (mmol/l)	4.9	4.7	4,5	0.08	0.274	0.492	0.032
Creatinine (mmol/dl)	94.7^{a}	92.3^{ab}	87.3^{b}	2.44	0.000	0.000	0.392
Energy metabolites and antioxidant capacity							
Glucose (mmol/l)	2.5^{b}	3.3^{a}	3.9^{a}	0.04	0.048	0.666	0.959
NEFA (mmol/L)	0.60^{a}	0.54^{b}	0.45^{c}	0.08	0.001	0.001	0.376
Cholesterol (mg/dL)	122.3^{a}	114.6^{b}	101.8^{c}	5.01	0.053	0.092	0.362
TAC (mmol/L)	0.17^{c}	0.23^{b}	0.35^{a}	0.02	0.001	0.197	0.236

MOLM: *Moringa oleifera* leaf meal, IgG: immunoglobulin G, g: gram, l: litre, mmol: millimole, dl: decilitre, TAC: total antioxidant capacity, NEFA: Non-esterified fatty acid

Discussion

Moringa leaf powder contains high levels of flavonoids, vitamin E, A and selenium, which could have contributed to the observed high antioxidant activity in the current study synergistically, as reported elsewhere (Falowo *et al*., 2016). Babiker *et al*. (2017) and Gopalakrishnam *et al*. (2016) reported that positive *Moringa* effects on milk yield were associated with a higher intake of the plant. Supplementation with phytobiotic-rich plants can stimulate the secretion of digestive fluids, which cause a shift in rumen fermentation, promoting the circulation of blood cholesterol and glucose, which reduce lipid mobilization (Kumar *at al*., 2017).

Likewise, in the current study, *Moringa oleifera* increased milk fat, TS and non-solid fat (Kholif *et al*., 2015). The observed high fat content in milk could be attributed to increased fermentation efficiency, enhanced by phytochemical-rich *Moringa* in conjunction with amino acids and P, Ca, and Mg in *M. oleifera* leaves, which are essential for high milk synthesis, as observed with other plants (Hashemzadeh-Cigari *et al*., 2014; Gopalakrishnan *et al*., 2016).

Milk yield and quality are critical determinants of a cow's dairy value (Politis, 2012). In the current study, the absorption of by-products of phenolic digestion could have reduced inflammation caused by stress. Low SCC is a benchmark in the milk value chain. The inhibition of pro-inflammatory cytokine synthesis suppresses cellular factor *k*B activation in immune cells (Zhan *et al*., 2016). Milk that is higher in antioxidants has a higher likelihood of reduced spoilage, therefore a longer shelf life (Aguiar *et al*., 2014). Antioxidants also stimulate lactoferrin activity in milk, which binds iron and inhibits iron-induced lipid peroxidation and hydrolysates (Gutterdge *et al*., 1981). This reaction prevents lipid peroxidation, hence promote better milk quality. High energy levels from elevated blood glucose level could have contributed to the lower non-esterified fatty acids levels observed in the current study.

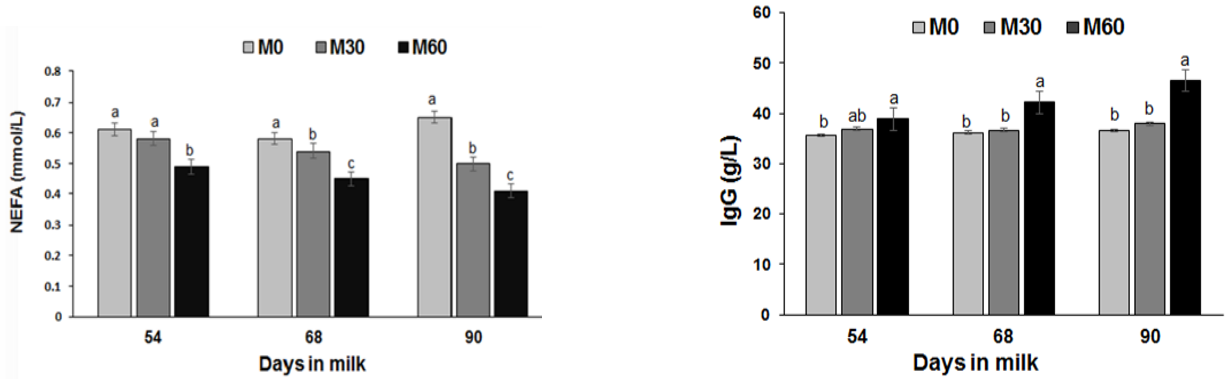

Figure 3 Blood non-esterified fatty acids in Jersey cows as affected by the micro-supplementation of *Moringa oleifera* leaf meal. Means with superscripts [abc] differ (P <0.05).

Normal cell metabolism, lactation and thermal stress produce free radicals (ROS), which cause oxidative stress if there is no mitigation strategy (Sejian *et al*., 2013). The observed increased STAC with *Moringa* supplementation indicates suppressed oxidative stress. Catalase activities and glutathione peroxidase and reduced Malondialdehyde (MDA) (Zhan *et al*., 2017) were the result of high phenolic activity. The reduced plasma creatinine concentration observed in cows supplemented with *M. oleifera* suggests an elevated mobilization of skeletal muscle protein (Bruckmaier *et al*., 1998). Creatinine levels often rise during negative energy balance, as more creatinine is burned to produce energy. The same trend was observed in MUN, which is positively correlated with creatinine, as noted by Nozad *et al*. (2012).

The unaffected globulins across the treatments could be explained by the increased albumin concentration that is stimulated by flavonoids such as quercetin, myricetin, and kaempferol (Papadopoulou *et al*., 2005) found in *M. oleifera* leaves (Pakade *et al*., 2013). Nfambi *at al*. (2015) also reported positive immunomodulatory effects owing to increased white blood cells, lymphocytes, and neutrophils levels. *Moringa* supplementation had minimal effects on blood urea levels, which indicates improved utilization of dietary nitrogen (Kumar *at al*., 2017; Nousiainen *et al*., 2004).

Conclusion

Supplementing *Moringa oleifera* leaf meal to lactating Jersey cows stimulated blood energy-related metabolites and serum antioxidant capacity, consequently improving milk quality with an associated reduction in oxidative stress.

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Authors' contributions

TWK conceptualised the idea and participated in the study design, data collection, laboratory and statistics analyses, and manuscript writing. UM was involved in constructive revision of manuscript. MCM participated in statistics analysis. FVN-C was involved in interpretation of the data and constructive revision of the manuscript.

Conflict of interest declaration

The authors have no competing interests to declare.

Reference

Aguiar, S.C., Cottica, S.M., Boeing, J.S., Samensari, R.B., Santos, G.T., Visentainer, J.V. & Zeoula, L.M., 2014. Effect of feeding phenolic compounds from propolis extracts to dairy cows on milk production, milk fatty acid composition, and the antioxidant capacity of milk. Anim. Feed Sci. Tech. 193, 148-154.

AOAC., 1990. Official Methods of Analysis. 15th edition. Association of Official Analytical Chemists, Washington, DC.

Babiker, E.E., Juhaimi, F.A.L., Ghafoor, K. & Abdoun, K.A., 2017. Comparative study on feeding value of Moringa leaves as partial replacement for alfalfa hay in ewes and goats. Livest. Sci. 195, 21-26.

Babiker, E.E., Juhaimi, F.A.L., Ghafoor, K., Mohamed, H.E. & Abdoun, K.A., 2016. Effect of partial replacement of alfalfa hay with Moringa species leaves on milk yield and composition of Najdi ewes. Trop. Anim. Health Pro. 48, 1427-1433.

Bamdad, F., Kadivar, M. & Keramat, J., 2006. Evaluation of phenolic content and antioxidant activity of Iranian caraway in comparison with clove and BHT using model systems and vegetable oil. Int. J. Food Sci. Tech. 41, 20-27.

Barkema, H.W., Von Keyserlingk, M.A.G., Kastelic, J.P., Lam, T.J.G.M., Luby, C., Roy, J.P., LeBlanc, S.J., Keefe, G.P. & Kelton, D.F., 2015. Invited review: Changes in the dairy industry affecting dairy cattle health and welfare. J. Dairy Sci. 98, 7426-7445.

Bouwstra, R.J., Nielen, M., Stegeman, J.A., Dobbelaar, P., Newbold, J.R., Jansen, E.H.J.M. & Van Werven, T., 2010. Vitamin E supplementation during the dry period in dairy cattle. Part I: Adverse effect on incidence of mastitis postpartum in a double-blind randomized field trial. J. Dairy Sci. 93, 5684-5695.

Bruckmaier, R.M., Gregoretti, L., Jans, F., Faissler, D. & Blum, J.W., 1998. Longissimus dorsi muscle diameter, backfat thickness, body condition scores and skinfold values related to metabolic and endocrine traits in lactating dairy cows fed crystalline fat or free fatty acids. Transbound. Emerg. Dis. 45, 397-410.

Cohen-Zinder, M., Leibovich, H., Vaknin, Y., Sagi, G., Shabtay, A., Ben-Meir, Y. & Miron, J., 2016. Effect of feeding lactation cows with ensiled mixture of Moringa oleifera, wheat hay and molasses, on digestibility and efficiency of milk production. Anim. Feed Sci. Tech. 211, 75-83.

De, K., Pal, S., Prasad, S. & Dang, A.K., 2014. Effect of micronutrient supplementation on the immune function of crossbred dairy cows under semi-arid tropical environment. Trop. Anim. Health Prod. 46.203-211.

Deacon, A.C. & Dawson, P.J., 1979. Enzymic assay of total cholesterol involving chemical or enzymic hydrolysis – a comparison of methods. Clin. Chem. 25, 976-984.

Falowo, A.B., Muchenje, V., Hugo, C.J. & Charimba, G., 2016. In vitro antimicrobial activities of Bidens pilosa and Moringa oleifera leaf extracts and their effects on ground beef quality during cold storage. CYTA-J. Food, 14, 541-546.

Gessner, D.K., Gröne, B., Rosenbaum, S., Most, E., Hillen, S., Becker, S. & Eder, K., 2015. Effect of a negative energy balance induced by feed restriction in lactating sows on hepatic lipid metabolism, milk production and development of litters. Arch. Anim. Nutr. 69, 399-410.

Gopalakrishnan, L., Doriya, K. & Kumar, D.S., 2016. Moringa oleifera: A review on nutritive importance and its medicinal application. Food Sci. Hum. Well. 5, 49-56.

Gutteridge, J.M.C., Paterson, S.K., Segalt, A.W. & Halliwellt, B., 1981. Inhibition of lipid peroxidation by the iron-binding protein lactoferrin. Biochem. J. 199, 259-261.

Hashemzadeh-Cigari, F., Khorvash, M., Ghorbani, G.R., Kadivar, M., Riasi, A. & Zebeli, Q., 2014. Effects of supplementation with a phytobiotics-rich herbal mixture on performance, udder health, and metabolic status of Holstein cows with various levels of milk somatic cell counts. J. Dairy Sci. 97, 7487-7497.

Johnson, M.J. & Peters, J.P., 1993. Technical note: an improved method to quantify non-esterified fatty acids in bovine plasma. J. Ani. Sci., 71:753-756

Jungbauer, A. & Medjakovic, S., 2014. Phytoestrogens and the metabolic syndrome. J. Ster. Bio. Mole. Bio. 139, 277-289.

Kekana, T.W., Nherera-Chokuda, F.V., Muya, M.C., Manyama, K.M., & Lehloenya, K.C., 2018. Milk production and blood metabolites of dairy cattle as influenced by thermal-humidity index. Trop. Anim. Health Pro. 1-4.

Kholif, A.E., Morsy, T.A., Gouda, G.A., Anele, U.Y. & Galyean, M.L., 2016. Effect of feeding diets with processed Moringa oleifera meal as protein source in lactating Anglo-Nubian goats. Anim. Feed Sci. Tech. 217, 45-55.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S. & Cosic, V., 2001. Method for the measurement of antioxidant activity in human fluids. J. Clin. Path. 54, 356-361.

Kumar, A., Mosa, K. A., Ji, L., Kage, U., Dhokane, D., Karre, S. & Pathania, N., 2017. Metabolomics-assisted biotechnological interventions for developing plant-based functional foods and nutraceuticals. Crit. Rev. Food Sci., 1-17.

Mbikay, M., 2012. Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review. Front. Pharmacol. 3, 24.

Moyo, B., Masika, P.J., Hugo, A. & Muchenje, V., 2011. Nutritional characterization of Moringa (*Moringa oleifera* Lam) leaves. Afr. J. Biotechnol. 10, 12925-12933.

Nfambi, J., Bbosa, G.S., Sembajwe, L.F., Gakunga, J. & Kasolo, J.N., 2015. Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in Wistar albino rats. J. Basic clin. Physiol. 26, 603-611.

Nousiainen, J., Shingfield, K.J. & Huhtanen, P., 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. J. Dairy Sci. 87, 386-398.

Nozad, S., Ramin, A., Moghadam, A., Asri-Rezaei, S., Babapour, A. & Ramin, S., 2012. Relationship between blood urea, protein, creatinine, triglycerides and macro-mineral concentrations with the quality and quantity of milk in dairy Holstein cows. Vet. Res. 3, 55 - 59

Pakade, V., Cukrowska, E. & Chimuka, L., 2013. Comparison of antioxidant activity of Moringa oleifera and selected vegetables in South Africa. S. Afr. J. Sci. 109, 1–5.

Papadopoulou, A., Green, R.J., & Frazier, R.A., 2005. Interaction of flavonoids with bovine serum albumin: A fluorescence quenching study. J. Agr. Food Chem. 53, 158-163.

Politis, I., Theodorou, G., Lampidonis, A.D., Kominakis, A. & Baldi, A., 2012. Oxidative status and incidence of mastitis relative to blood α-tocopherol concentrations in the postpartum period in dairy cows. J. Dairy Sci., 95, 7331-7335.

Rizzo, A., Pantaleo, M., Mutinati, M., Minoia, G., Trisolini, C., Ceci, E. & Sciorsci, R.L., 2013. Blood and milk oxidative status after administration of different antioxidants during early postpartum in dairy cows. Res. Vet. Sci. 95, 1171-1174.

SAS. 2009. User's guide: Statistics, version 9.1. SAS Inst. Inc. Cary, NC.

Sejian, V., 2013. Climate change: Impact on production and reproduction, adaptation mechanisms and mitigation strategies in small ruminants: A review. Indian J. Small Rumin. 19, 1-21.

Sordillo, L.M. & Raphael, W., 2013. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. Vet. Clin.: Food Ani. Pract. 29, 267-278.

Tainton, N.M., 1999. The ecology of the main grazing lands of South Africa. In: N.M. Tainton (ed). Veld management in South Africa. University of Natal Press, Pietermaritzburg, South Africa. pp. 48.

Tietz, N.W., 1995. Clinical guide to laboratory tests. WB Saunders, Philadelphia.

Ule, E., 2015. Measurement of the nonlinear refractive index by Z-scan technique. University of Ljubljana, Slovenia, 4-5.

Van den Berg, R., Haenen, G.R., Van den Berg, H. & Bast, A.A.L.T., 1999. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chem. 66, 511-517.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583.

Yatoo, M.I., Deepa, P.M., Mandal, R.S.K., Sharma, B., Mendiratta, S.K., Patel, B.H.M. & Dimri, U., 2015. Prevalence of subclinical diabetes in a commercial flock of dairy goats in India and its interaction with milk quality. Small Rum. Res. 132,1-11.

Zebeli, Q., Dunn, S.M. & Ametaj, B.N., 2011. Perturbations of plasma metabolites correlated with the rise of rumen endotoxin in dairy cows fed diets rich in easily degradable carbohydrates. J. Dairy Sci. 94, 2374-2382.

Zhan, J., Liu, M., Su, X., Zhan, K., Zhang, C. & Zhao, G., 2017. Effects of alfalfa flavonoids on the production performance, immune system, and ruminal fermentation of dairy cows. Asian-Australas. J. Anim. Sci. 30, 1416.