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Total phenolic content, non-tannin phenols, tannins and DPPH radical scavenging activity in concentrate mixtures containing varying levels of *Moringa oleifera* (Moringa) leaves

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Abstract
A study was conducted to evaluate the effect of inclusion of moringa leaves at varying levels in concentrate mixture on total phenols and antioxidant activity. The antioxidant capacity of moringa (MOR) leaves and their inclusion in the concentrate mixtures at varying levels were evaluated *in vitro*. The control concentrate mixture was prepared with maize grain, de-oiled rice bran, cotton seed cake and soybean meal as major ingredients. The other 7 iso-nitrogenous (20% CP) concentrate mixtures were prepared by partially replacing de-oiled rice bran and cotton seed cake with MOR at varying levels (5, 10, 15, 20, 25, 30 and 35%). The total phenolic content (TPC) gradually increased (p<0.01) from 3.87 to 19.30 mg of gallic acid equivalent (GAE) per g and from 4.13 to 21.36 mg of tannic acid equivalent (TAE) per g with inclusion of MOR leaves from 0 to 35% in concentrate mixtures. The non-tannin phenols (NTAP) and tannins (TA) increased (p<0.01) from 2.79 to 8.35 mg of TAE per g and 1.34 to 14.23 mg of tannins per g. Similarly, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (%) increased (p<0.01) from 0 to 30% (7.29 to 78.09) with inclusion of MOR leaves in concentrate mixtures and the activity at 35% (79.50) inclusion was comparable with that of 30% (78.09). The increase in TPC, NTAP, TA and DPPH activity in concentrate mixtures was due to higher content of these components in MOR leaves.

Keywords: Moringa leaves, total phenolic content, tannins, non-tannin phenols, DPPH activity

Introduction
Moringa species are multipurpose trees from non-leguminous group. They are fast growing trees of economic and industrial importance and a potential source of animal feed. India is the largest producer of moringa with an annual production of 1.1 to 1.3 million tons of fruit pods (Patel *et al.*, 2010) [17]. *Moringa oleifera* foliage has been reported to be a potential cheap source of protein for animal feeding (Sarwatt *et al.*, 2004) [19]. Kholif *et al.* (2015) [12] reported moringa as a protein feed, containing 241-277 g/kg of crude protein, among which about 47% of bypass protein (Becker, 1995) [3], adequate amino acid profile (Sanchez-Machado *et al.*, 2010) and polyphenolics contents as antioxidant (Sreelatha and Padma, 2009) [22]. Compared to traditional protein feeds *viz.*, cotton seed cake, groundnut cake, sesame meal and soybean meal for ruminants *M. oleifera* is a cheaper protein ingredient (Kholif *et al.*, 2015) [12].
Similary, *Moringa oleifera*, like other tree fodders, has low levels of anti-nutritive compounds and high levels of naturally occurring antioxidants like vitamin C, tocopherols, flavonoids, and phenolic compounds (Soliva *et al.*, 2005; Mendieta-Araica *et al.*, 2011; Nouman *et al.*, 2014) [21, 14, 16]. Plants with bioactive compounds with antibacterial qualities, including as essential oils, saponins, and condensed tannins (Mendieta-Araica *et al.*, 2011; Nouman *et al.*, 2014) [14, 16], can be used to increase antioxidant status and protein alternative in livestock. The use of morigna leaf meal as a protein source and natural antioxidant for ruminants is not well understood. Recently, researchers are interested in using moringa leaf meal as a protein feed and natural antioxidant due to its encouraging outcomes, including increased feed utilisation and milk production in small ruminants (Kholif *et al.*, 2015) [12]. Hence, the present research was undertaken to estimate the total phenols, tannins and antioxidant activity in concentrate mixtures with increased inclusion of moringa leaves at varying levels.

Materials and Methods

The effect of inclusion of *Moringa oleifera* (moringa) leaves at 0, 5, 10, 15, 20, 25, 30 and 35% in the concentre mixtures were taken for *in vitro* antioxidant assay. The concentrate mixtures with various inclusion levels of moringa leaves were prepared in triplicate for each diet. A basal concentrate mixture (control, CON) was formulated with maize, de-oiled rice bran, cotton seed cake and soybean meal as major ingredients. The remaining 7 concentrate mixtures were formulated with *Moringa oleifera* leaves (MOR). The ingredient composition of the 8 concentrate mixtures containing *Moringa oleifera* (Moringa) leaves (MOR) at various inclusion levels is given in Table 1.

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity: The DPPH radical scavenging activity was determined according to the method described by Hossain and Shah (2015) [9] with slight modifications. The samples (1 g) were extracted in 20 ml cold solution of 3% oxalic acid in 8% glacial acetic acid and centrifuged at 15000 rpm at 4 °C for 10 minutes and supernatant was collected and used for assay. The DPPH (Sigma Aldrich) solution (0.2 mM) was prepared using 95% ethanol. Samples were analyzed using a microtitre plate using blank, positive, negative and test samples. The blank, positive, negative and test samples consists of 25 µl of distilled water, L-ascorbic acid, di-methyl sulphoxide and extract from concentrate mixture along with 250 µl of DPPH (0.2 mM). The samples were incubated in dark for 30 minutes and absorbance was measured at 517 nm using ELISA reader - µQuant (BioTek instruments). L-ascorbic acid (17.6 mg in 100 ml distilled water) was used as positive control and di-methyl sulphoxide as negative control.

Absorbance of control = Postive control OD – Negative control OD

The scavenging activity was calculated using the following formula and expressed in percent of radical scavenging activity. The determination of DPPH radical scavenging activity was carried out in triplicate and results were averaged.

Table 1: Ingredient composition (%) of concentrate mixtures containing varying levels of *Moringa oleifera* leaves (MOR)

Ingredient	CON[2]	MOR5[3]	MOR10[3]	MOR15[3]	MOR20[3]	MOR25[3]	MOR30[3]	MOR35[3]
Maize	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soybean meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
De-oiled rice bran	37.00	34.00	30.00	27.00	24.00	20.00	17.00	14.00
Cotton seed cake	21.00	19.00	18.00	16.00	14.00	13.00	11.00	9.00
Moringa oleifera leaves	0.00	5.00	10.00	15.00	20.00	25.00	30.00	35.00
Mineral and vitamin mixture[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calcite powder	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Salt	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]Mineral and vitamin mixture provided per kg diet: Calcium 2.5 g, Phosphorus 1.275 g, Magnesium 0.065 g, Iron 0.0175 g, Sulphur 0.092 g, Zinc 0.096 g, Copper 0.042 g, Manganese 0.015 g, Potassium 1.5 mg, Sodium 0.2 mg, Iodine 3.5 mg, Cobalt 1.5 mg, Vitamin B_6 0.2 mg, Vitamin A 7500 IU, Vitamin D_3 750 IU, Vitamin E 3 mg, Niacinamide 0.012 g.
[2]Control diet
[3]*Moringa oleifera* (Moringa) leaves included at 5, 10, 15, 20, 25, 30 and 35% in the concentrate mixture partially replacing de-oiled rice bran and cotton seed cake.

$$\text{DPPH Radical scavenging activity (\%)} = \frac{\text{(Abs. of control – Abs. of sample)}}{\text{Abs. of control}} \text{ X } 100$$

Total Phenolic Content – Gallic Acid Equivalent: The total phenolic content (TPC) was determined by the Folin–Ciocalteu method (Hossain and Shah, 2015) [9] with some modifications. About 200 mg of sample was treated with 10 ml diethyl ether containing 1% acetic acid, vortexed and centrifuged at 3000 rpm for 5 minutes to remove any pigments. The supernatant was carefully discarded. To the pellet, 10 ml of 70% aqueous acetone was added and incubated for 2 h at 30ºC using shaker. A standard curve was prepared using 0.00, 0.02, 0.04, 0.06, 0.08 and 0.10 ml of 0.1 mg/ml gallic acid stock solution and volume was made up to 0.5 ml with distilled water. Then transferred 0.5 ml of extracts to test tubes, later 0.25 ml of 1N Folin–Ciocalteu reagent was added to samples and standards followed by 1.25 ml of 20% sodium carbonate solution. The test tubes were vortexed and incubated at room temperature for 40 minutes and absorbance was recorded using spectrophotometer (UV-61 PCS, Metstar) at 650 nm. Total phenols as gallic acid equivalent (GAE mg/g) was calculated from calibration curve and expressed on DM basis.

Total Phenolic Content – Tannic Acid Equivalent: The total phenolic content (TPC) was determined by the Folin–Ciocalteu method (Makkar, 2003) [13] with some modifications. About 200 mg of samples were treated with 10 ml diethyl ether containing 1% acetic acid, vortexed and centrifuged at 3000 rpm for 5 minutes to remove any pigments. The supernatant was carefully discarded. To the pellet, 10 ml of 70% aqueous acetone was added and incubated for 2 h at 30ºC using shaker. A standard curve was prepared using 0.00, 0.02, 0.04, 0.06, 0.08 and 0.10 ml of 0.1 mg/ml tannic acid stock solution and volume was made up to 0.5 ml with distilled water. Then transferred 0.5 ml of extracts to test tubes, 0.25 ml of 1N Folin–Ciocalteu reagent was added to samples and standards followed by 1.25 ml of 20% sodium carbonate solution. The contents were vortexed and incubated at room temperature for 40 minutes and absorbance was recorded using spectrophotometer (UV-61 PCS, Metstar) at 725 nm. Total phenols as tannic acid equivalent (TAE mg/g) was calculated form calibration curve and expressed on DM basis.

Non-tannin Phenolic Content and Tannins: The non-tannin phenols was determined by the Folin–Ciocalteu method (Makkar, 2003) [13] with some modifications. Polyvinyl polypyrolidone (PVPP) binds tannins present in extract. Weighed 100 mg of PVPP (100 mg of PVPP is sufficient to bind 2 mg of tannin phenols) and transferred to test tube, to this 1 ml distilled water and 1 ml of tannin extract was added. Standard curve (tannic acid) as mentioned earlier using tannic acid and extracts were treated similarly as that of total phenols estimation for tannic acid equivalent and expressed as non-tannin phenols.

Tannins in samples were estimated using the formula
Total tannins (mg of TAE/g) = (Total tannin phenols) – (non-tannin phenols)
The data obtained were subjected to statistical analysis using software (SPSS, Version 17). One way analysis of variance through generalized linear model was used to analyse all the results. The treatment means were ranked using Duncan's multiple range test with a significance at $p<0.05$. All the statistical procedures were done as per Snedecor and Cochran (1994) [20].

Results and Discussion

The total phenolic content (GAE and TAE mg/g), non-tannin phenols (mg TAE/g), and tannins (mg/g) differed significantly ($p<0.01$) with variation in inclusion levels of moringa leaves in concentrate mixtures (Table 2). The total phenolic content in terms of gallic acid equivalent (GAE) (mg/g GAE) increased linearly ($p<0.01$) with increase in moringa leaves inclusion levels from 0 (CON) (3.87) to 35% (MOR35) (19.30). The GAE of CON, MOR5, MOR10, MOR15, MOR20, MOR25, MOR30 and MOR35 were in the order of MOR35 (19.30) > MOR30 (16.98) > MOR25 (16.10) > MOR20 (14.75) > MOR15 (11.81) > MOR10 (8.29) > MOR5 (6.53) > CON (3.87) concentrate mixtures. Similarly, the total phenolic content in terms of tannic acid equivalent (TAE) (mg/g TAE) was highest ($p<0.01$) in MOR25, MOR30 and MOR35 were 20.49, 20.22 and 21.36, respectively and was comparable among each other. The lowest total phenolic content was observed in CON (4.13) concentrate mixture. The TAE increased linearly ($p<0.01$) with increase in moringa leaves inclusion levels from 0 (CON) (4.13) to 25% (MOR25) (20.49). No increase in TAE was observed with increase in moringa leaves from 25 (20.49) to 35% (21.36) in concentrate mixtures.

The gradual increase in total phenolic content in concentrate mixtures with increased inclusion of moringa leaves could be due to high phenolic content in moringa leaves compared to other feed ingredients in concentrate mixtures as reported by various authors (Sreelatha and Padma, 2009 [22], Das *et al.*, 2012 [5], Moyo *et al.*, 2012 [15] and Belhi *et al.*, 2018) [4]. The total phenolic content observed in moringa leaves based concentrate mixtures in our study was higher than that reported by Babiker *et al.* (2016) [2] and Al-Juhaimi *et al.* (2020) [1] when moringa leaves was included at 25% in the total diet and the total phenol content value obtained was equivalent to that of 5% MOR leaves inclusion in concentrate mixture of present study, which could be due to difference in variety (Babiker *et al.*, 2016) [2] and maturity (Sreelatha and Padma, 2009) [22] of moringa leaves. The total phenolic content decreases with maturity (Sreelatha and Padma, 2009) [22] of leaves.

Table 2: Effect of inclusion of *Moringa oleifera* (moringa) leaves at varying levels in concentrate mixture on total phenolic content (GAE and TAE), non-tannin phenols and tannins assessed *in vitro*

Diet	Total phenolic content (TPC) mg GAE / g	Total phenolic content (TPC) mg TAE / g	Non-tannin phenols (NTAP) mg TAE / g	Tannins mg / g
CON[1]	$3.87^{h}\pm0.01$	$4.13^{f}\pm0.03$	$2.79^{g}\pm0.03$	$1.34^{g}\pm0.06$
MOR5[2]	$6.53^{g}\pm0.09$	$7.46^{e}\pm0.16$	$3.78^{f}\pm0.07$	$3.68^{f}\pm0.11$
MOR10[2]	$8.29^{f}\pm0.11$	$9.35^{d}\pm0.09$	$4.04^{ef}\pm0.11$	$5.31^{e}\pm0.17$
MOR15[2]	$11.81^{e}\pm0.38$	$13.68^{c}\pm0.48$	$4.40^{e}\pm0.03$	$9.28^{d}\pm0.46$
MOR20[2]	$14.75^{d}\pm0.09$	$17.31^{b}\pm0.23$	$5.38^{d}\pm0.24$	$11.93^{c}\pm0.24$
MOR25[2]	$16.10^{c}\pm0.08$	$20.49^{a}\pm0.25$	$6.26^{c}\pm0.16$	$14.23^{a}\pm0.10$
MOR30[2]	$16.98^{b}\pm0.15$	$20.22^{a}\pm0.52$	$6.78^{b}\pm0.20$	$13.44^{ab}\pm0.45$
MOR35[2]	$19.30^{a}\pm0.46$	$21.36^{a}\pm1.10$	$8.35^{a}\pm0.29$	$13.01^{bc}\pm0.82$
SEM	1.075	1.300	0.360	0.975
P value	0.0001	0.0001	0.0001	0.0001

Each value is an average of three observations
[abcd]Means with different superscripts in a column differ significantly: P≤0.01
GAE- Gallic acid equivalent, TAE- Tannic acid equivalent
[1]Control diet (0% moringa leaves)
[2]*Moringa oleifera* (moringa) leaves included at 5, 10, 15, 20, 25, 30 and 35% in the concentrate mixture partially replacing de-oiled rice bran and cotton seed cake.
[3]*Moringa oleifera* (moringa) leaves
SEM: Standard Error Mean; P value: Probability value.

Table 3: Effect of inclusion of *Moringa oleifera* (moringa) leaves at varying levels in concentrate mixture on DPPH radical scavenging activity assessed *in vitro*

Diet	DPPH radical scavenging activity (%)
CON[1]	$7.29^{f}\pm0.71$
MOR5[2]	$36.57^{e}\pm1.65$
MOR10[2]	$41.72^{d}\pm0.11$
MOR15[2]	$64.58^{c}\pm1.04$
MOR20[2]	$71.29^{b}\pm0.97$
MOR25[2]	$73.91^{b}\pm0.63$
MOR30[2]	$78.09^{a}\pm0.21$
MOR35[2]	$79.50^{a}\pm1.14$
SEM	5.018
P value	0.0001

Each value is an average of three observations
[abcd]Means with different superscripts in a column differ significantly: $p\leq0.01$
DPPH- 2,2-diphenyl-1-picrylhydrazyl
[1]Control diet (0% moringa leaves)
[2]*Moringa oleifera* (moringa) leaves included at 5, 10, 15, 20, 25, 30 and 35% in the concentrate mixture partially replacing de-oiled rice bran and cotton seed cake.
[3]*Moringa oleifera* (moringa) leaves
SEM: Standard Error Mean; P value: Probability value.

The non-tannin phenols (NTAP) (mg TAE/g) was highest (p<0.01) in MOR35 (8.35) and lowest was observed in CON (2.79) concentrate mixtures that increased linearly with increase in moringa leaves inclusion in concentrate mixtures from 0 to 35%. The NTAP (mg TAE/g) of CON, MOR5, MOR10, MOR15, MOR20, MOR25, MOR30 and MOR35 were in the order of MOR35 (8.35) > MOR30 (6.78) > MOR25 (6.26) > MOR20 (5.38) > MOR15 (4.40) ≥ MOR10 (4.04) ≥ MOR5 (3.78) > CON (2.79) concentrate mixtures. The tannins (TA) (mg/g) was highest (p<0.01) in MOR25 (14.23) and MOR30 (13.44) and lowest was observed in CON (1.34) concentrate mixtures. The TA content increased linearly (p<0.01) with increase in moringa leaves inclusion levels from 0 (CON) (1.34) to 25% (MOR25) (14.23). No change on TA content was observed from 25 (14.23) to 30% (13.44) and from 30 (13.44) to 35% (13.01), while the TA content in MOR30 was comparable with MOR25 and MOR35.

The non-tannin phenols and tannins also showed similar trend as that of total phenols in concentrate mixtures with varying levels of moringa leaves. The results showed that the moringa leaves inclusion had lower content of tannin (below 4%), an anti-nutritional substance. Similar results were reported by Ferreira *et al.* (2008) [7] who observed low content of tannins (12 mg/g) in *M. oleifera* leaves. Further, Teixeira *et al.* (2014) [23] also reported 20.6 mg/g total tannin content in *M. oleifera* leaves and Jadhav *et al.* (2019) [10] reported 19.6 mg/g and 39.6 mg/g of total tannins in *M. oleifera* on DM basis grown in two different regions, which was comparable with our findings.

The DPPH assay has been widely used to evaluate the free radical scavenging ability of various plant extracts. The DPPH radical scavenging activity increased linearly (p<0.01) with increase in moringa leaves inclusion levels from 0 (CON) (7.29) to 20% (MOR20) (71.29) and further increase was observed from MOR25 (73.91) to MOR30 (78.09). No increase in DPPH radical scavenging activity was observed with increase in moringa leaves from 30 (78.09) to 35% (79.50) in concentrate mixture and similarly, the activity between 20 (71.29) and 25% (73.91) inclusion level was comparable. Similarly, Sreelatha and Padma (2009) [22], Das *et al.* (2012) [5], Moyo *et al.* (2012) [15] and Belhi *et al.* (2018) [4] reported concentration dependent increase in DPPH radical scavenging activity (%) with increasing concentration of moringa leaf extract which was in agreement with our findings, that DPPH activity increased with increase in inclusion level of moringa in concentrate mixtures. The high DPPH activity in the concentrate mixtures with higher inclusion levels of moringa is due to increase in total phenols, non-tannins phenols and tannin content in these concentrate mixtures. The antioxidant plant activities of moringa correlate with total polyphenols (Moyo *et al.*, 2011, Moyo *et al.*, 2012 [15], Belhi *et al.*, 2018 [4] Al-Juhaimi *et al.*, 2020) [1], flavonoids, flavonols, proanthocyanidin (Moyo *et al.*, 2012) [15], total tannins and saponins (Jelali and Salem, 2014) [11]. Corroborating with the present findings, Gaafar *et al.* (2016) [8] observed that the antioxidant power in moringa was well correlated with the richness of the plant in phenol compounds such as ellagic acid (41.1%), quercetin (6.3%) and benzoic acid (26.01%).

Conclusions

In vitro screening of various inclusion levels of moringa leaves in concentrate mixtures for their effects on total phenols, non-tannin phenols and tannin phenols suggests that these contents increase with increased inclusion of moringa leaves. Similarly the DPPH radical scavenging activity (%) also increased with increased inclusion of moringa leaves in concentrate mixtures. Inclusion of moringa leaves in concentrate mixtures at higher levels is having higher levels of TPC, NTPC, tannins and DPPH radical scavenging activity (%) thus may help in improving antioxidant content of feed and thus may enhance antioxidant status of animals when included in rations and further may enhance stress management. Moringa leaves are thus potential alternative for the synthetic antioxidants in the animal feeding system and can economize the livestock feeding and consumer acceptance of animal products.

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References

1. Al-Juhaimi FY, Alsawmahi ON, Abdoun KA, Ghafoor K, Babiker EE. Antioxidant potential of moringa leaves for improvement of milk and serum quality of Aardi goats. South African Journal of Botany. 2020;129:134-137.
2. Babiker EE, Al-Juhaimi F, Ghafoor K, Mohamed HE, Abdoun KA. Effect of partial replacement of alfalfa hay with moringa species leaves on milk yield and composition of Najdi ewes. Tropical Animal Health Production. 2016;48:1427-1433.
3. Becker K. Studies on utilization of *Moringa oleifera* leaves as animal feed. In: institute for animal production in the tropics and subtropics. University of Hohenheim, Stuttgart. 1995;480:15-20.
4. Belhi M, Selmi H, Abbes C, Jedidi S, Tibaoui G, Rouissi H. The antioxidant activity and the ruminal fermentation parameters of *Moringa oleifera* L. among sheep and goats. Journal of Agriculture and Environmental Science. 2018;7(1):83–92.
5. Das AK, Rajkumar V, Verma AK, Swarup D. *Moringa oleiferia* leaves extract: a natural antioxidant for retarding lipid peroxidation in cooked goat meat patties. International Journal of Food Science and Technology. 2012;47:585-591.
6. Duncan DB. Multiple range and multiple F tests. Biometrics. 1995;11(1):1-42.
7. Ferreira PMP, Farias DF, Oliveira JT, de A, Carvalho A, de F U. *Moringa oleifera*: Bioactive compounds and nutritional potential. Revista de Nutricao. 2008;21(4):431-437.
8. Gaafar M, Alaa A, Eman A, Ibrahim A, Mohsen S, Asker Ahmed F, *et al.* Characterization of polyphenols, polysaccharides by HPLC and their antioxidant, antimicrobial and antiinflammatory activities of defatted moringa (*Moringa oleifera* L.) meal extract. International Journal of Pharmaceutical and Clinical Research. 2016;8(6):565-573.
9. Hossain MA, Shah MD. A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant Merremia borneensis. Arabian Journal of Chemistry. 2015;8:66-71.
10. Jadhav RV, Chaudhary LC, Agarwal N, Kala A, Kamra

DN. *In vitro* assessment of tropical tree leaves as modulators of rumen fermentation. Animal Nutrition and Feed Technology. 2019;19:443-454.
11. Jelali R, Salem HB. Daily and alternate day supplementation of *Moringa oleifera* leaf meal or soyabean meal to lambs receiving oat hay. Livestock Science. 2014;168:84-88.
12. Kholif AE, Gouda GA, Morsy TA, Salem AZM, Lopez S, Kholif AM. *Moringa oleifera* leaf meal as a protein source in lactating goat's diets: Feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile. Small Ruminant Research. 2015;129:129-137.
13. Makkar HPS. Quantification of Tannins in Tree and Shrub Foliage. Springer, Netherlands; c2003. p. 1-51.
14. Mendieta-Araica B, Sporndly R, Sanchez NR, Sporndly E. Moringa (*Moringa oleifera*) leaf meal as a source of protein in locally produced concentrates for dairy cows fed low protein diets in tropical areas. Livestock Science. 2011;137:10-17.
15. Moyo B, Oyedemi S, Masika PJ, Muchenje V. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. Meat Science. 2012;91:441-447.
16. Nouman W, Basra SMA, Siddiqui MT, Yasmeen A, Guli T. Alcayde MAC. Potential of *Moringa oleifera* L. as livestock fodder crop: A review. Turkish Journal of Agriculture and Forestry. 2014;38:1-14.
17. Patel S, Thakur AS, Chandy A, Manigauha A. *Moringa oleifera*: A review of there medicinal and economical importance to the health and nation. Drug Invention Today. 2010;2:339-342.
18. Sanchez-Machado D, Nunez-Gastelum J, Reyes-Moreno C, Ramírez-Wong B, Lopez-Cervantes J. Nutritional quality of edible parts of *Moringa oleifera.* Food Analytical Methods. 2010;3:175-180.
19. Sarwatt SV, Milangha MS, Lekule FP, Madalla N. *Moringa oleifera* and cottonseed cake as supplements for smallholder dairy cows fed Napier grass. Livestock Research for Rural Development. 2004;16(6):38.
20. Snedecor GW, Cochran WG. Statistical Methods. Oxford and IBM Publications New Delhi; c1994. p. 265.
21. Soliva CR, Kreuzer M, Foidl N, Foidl G, Machmuller A, Hess HD. Feeding value of whole and extracted *Moringa oleifera* leaves for ruminants and their effects on ruminal fermentation *in vitro*. Animal Feed Science and Technology. 2005;118:47-62.
22. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods for Human Nutrition. 2009;64:303-311.
23. Teixeira EM, Carvalho MR, Neves VA, Silva MA, Arantes-Pereira L. Chemical characteristics and fractionation of proteins from *Moringa oleifera* Lam. Leaves. Food Chemistry. 2014;47:51-54.