Nutritional evaluation of *moringa oleifera* fodder in comparison with *trifolium alexandrinum* (berseem) and impact of feeding on lactation performance of cows.

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Abstract: This study was carried out on two phases, the 1st phase was to evaluate nutrients, minerals, essential amino acids contents and *in-sacco* effective degradability of dry matter (DM) and crude protein (CP) of fresh bereseem (*Trifolium alexandrinum*) against *Moringa oleifera* forage. The 2nd phase was on farm evaluation of three rations consisted of (DM basis): 60% concentrate feed mixture with 40% berseem (R_1), 40% moringa (R_2) and 20% berseem + 20% moringa (R_3). Experimental rations were evaluated on sheep for nutrients digestibility, dietary nitrogen utilization and rumen fermentation activity. The three tested rations (R_1, R_2 and R_3) were evaluated on 15 multiparous cross bred Friesian cows for milk production and composition. Animals after 60 days of parturition were randomly blocked by weight and previous milk records into three equal groups for a feeding period of 12 weeks, where milk production was daily recorded and milk composition was by-weekly determined. Blood samples were collected twice at end of the feeding period. The results of the comparative chemical analysis showed that moringa had higher crude protein, total phenolic compounds, condensed tannins essential amino acids and mineral contents Ca, P, Mg, Fe, Mn and Zn than Berseem. Ruminal kinetics showed higher ($P<0.05$) values of potentially degradable and effective degradability of DM for Moringa, while corresponding values of CP were higher ($P< 0.05$) for berseem than moringa. Rations contained Moringa had higher nutrients digestibility values and better nitrogen utilization ($P < 0.05$) than R_1 (contained only berseem). Moringa rations significantly ($P <0.05$) increased ruminal VFAs, microbial yield and acetic acid concentration. Feeding cows on moringa fodder showed similar DM intake as for those fed berseem, suggesting that both forages had the same degree of palatability. Moringa rations increased ($P<0.05$) the daily yield of 4% FCM by 25% for R_2 (moringa ration) and 16% for R_3 (moringa + berseem) than that fed R_1 (berseem ration). Milk constituents including total solids, solids not fat, fat, protein and ash were increased ($P<0.05$) with Moringa rations. However, milk lactose did not significantly influenced by changing forage type. Feed conversion (Kg DM intake / kg FCM) was improved ($P<0.05$) with moringa rations (R_2 and R_3) than berseem ration (R_1) (1.02 and 1.08 vs. 1.24). Feeding moringa rations was associated with higher ($P<0.05$) blood glucose concentration and enzymatic antioxidants activity (GSH-Px, CAT and SOD) with lower ($P< 0.05$) blood cholesterol and urea contents than animals fed all berseem ration (R_1). Under the conditions of this study, it's fair to conclude that, *Moringa oleifera* is palatable and highly nutritious fodder with antioxidant properties which was reflected on the improvement of milk yield and composition. Therefore, the partial or complete replacement of berseem with moringa is highly recommended in the feeding practices of dairy cows.

[Mohamed S. Khalel; Amr M. Shwerab; Ayman A. Hassan; Mohamed H.Yacout; Alaa Y.El-Badawi and Mona S. Zaki. **Nutritional evaluation of *moringa oleifera* fodder in comparison with *trifolium alexandrinum* (berseem) and impact of feeding on lactation performance of cows.** *Life Sci J* 2014;11(10):1040-1054]. (ISSN:1097-8135). http://www.lifesciencesite.com. 158

Keywords: *Moringa oleifera*, Friesian cows, rumen fermentation and kinetics, blood constituents, milk production, composition.

1. Introduction

In Egypt, there is a great developing gab between demands and available resources of animal protein. The national animal protein basket (milk, egg and red and white meat) is providing nearly 18.4g/day/cap., where milk provides 5.2g protein/day/cap. (**Ministry of Agriculture, 2010**). Comprising 28% of the whole animal protein annually produced. The policy of animal protein development aims to reach 24g protein / day/cap. by year 2030, where the annual milk production is forecasted to reach 6.000,000 ton. Mean while high prices of concentrate feeds (oil seeds meal and gains) and in sufficiency of good quality green fodders all over the year are the two main factors constrain animal production development. Berseem (*Trifolium alexandrinum*) is the traditional Egyptian green fodder in during winter season (from Nov. up end of May). About 2.000.000 feddan (Feddan = 4200m^2) are annually cultivated with berseem for ruminants feeding. Abundant amounts of nearly 40,

000,000 ton of berseem forage are available during winter while green fodders are scarcely available during summer season. Limited level of irrigated water and occupation of agricultural lands with traditional summer food crops (rice, corn .. etc.) are the two main constrains make sufficient amount of green fodders un available in reasonable cost during summer season. So, there is a bad need to find type of perennial fodder plant capable to grow particularly, in the sandy soil of the newly reclaimed lands.One of the most interesting trees is *Moringa oleifera* Lam (syns. Moringa pterygosperm, family Moringaceae) which is a native tree in Himalaya and currently spread almost world-wide. This tree grows throughout tropics and sub-tropical regions. It is one of the most widely used species for fodder (**Bakhashwain *et al.*, 2010**). It can grow in all types of soils and can tolerate dry seasons lasting up to 6 months (**Mendieta-Araica *et al.*, 2013**). The total dry matter (DM) yield from moringa is up to 24 ton ha^{-1} $year^{-1}$ (**Reyes-Sanchez *et al.*, 2006$_a$**). Furthermore, *Moringa oleifera* Lam is a non-leguminous multipurpose tree with a high crude protein in the leaves (251g/kg DM) and negligible content of tannis and other anti-nutritional compounds (**Makkar and Becker, 1996 and Gidamis *et al.*, 2003**). Moringa plant contains significant amounts of vitamins A, B, and C in the foliage with a good profile of amino acids (**Ferreira *et al.*, 2008 and Mendieta - Araica *et al.*, 2011**). As fresh forage, moringa has been included in the diets of many different animals. Positive effects on the feeding behavior in goats (**Manh *et al.*, 2005**) and on the growth rate in sheep (**Ben Salem and Makkar, 2009**); and had favorable production results with dairy cows (**Reyes-Sanchez *et al.*, 2006$_b$**). Moringa leaves and green pods are used as vegetables by humans and offer well an alternative source of protein to ruminants. Moreover, its twigs are very palatable to ruminants and have appreciable crude protein levels (**Kaijage *et al.*, 2003**). During the last five years, great attention has been given to moringa forage by many Egyptian animal nutritionists to overcome the difficulty of green fodder shortage particularly during summer season; however the high cost of imported moringa seeds is still constraining its cultivation development. So, this study was conducted to investigate the impact of feeding moringa fodder in partial or complete replacement of berseem forage in the feeding practices of dairy cows.

2. Materials and Methods

Feeds and tested rations:

The present study was carried out at Al- Noubaria Experimental Station affiliates Animal Production Research Institute, Agricultural Research Center, Giza, Egypt, in corporation with a private moringa farm located in Al-Noubaria province (about 180 km North Western Cairo City).The study was conducted on two phases, the 1st phase was to explore chemical composition, minerals content, essential amino acids profile and *in-sacco* DM and CP degradability of moringa and berseem. The 2nd phase was to investigate the nutritional impact of three tested rations of partial or complete replacement of berseem with moringa on sheep for nutrients digestibility, dietary nitrogen utilization and rumen fermentation activity. The three tested rations were evaluated on milk production and composition and biochemical constituents of blood of dairy cows. Experimental fodders of 3rd cut berseem (*Trifolium alexandrinum*) and moringa (*Moringa oleifera Lam.*) were daily collected and brought to the animal station at 6.00 a.m. Three experimental rations based on changing the roughage type were fed on DM basis as: R_1 60% concentrate feed mixture (CFM) + 40% fresh berseem, R_2 60% CFM + 40% fresh moringa and R_3 60% CFM + 20% berseem + 20% moringa. Calculated feeding value of the CFM was 62% total digestible nutrients (TDN) and 16% crude protein (CP). Proximate chemical analysis and cell-wall constituents of CFM were determined according to the standard methods of **A.O.A.C. (1995) and Van Soest *et al.* (1991)**, respectively.

Table (1): Chemical composition and cell-wall constituents of the CFM.

Moisture %	DM Composition, %						Cell-wall constituents, %				
	OM	CP	EE	CF	NFE	Ash	NDF	ADF	ADL	Hemi-cellulose	Cellulose
12.42	91.08	16.02	2.92	12.31	59.83	8.92	31.02	20.07	2.88	10.95	17.19

The concentrate feed mixture in cubes was formulated of: 31% ground yellow corn, 8% soybean meal. 17% undecorticated cotton seed meal, 18% wheat bran, 18% rice bran, 4.5% canemolasses, 2% lime stone, 1% sodium chloride and 0.5% mineral mixture. OM= organic matter, CP= crude protein, EE= ether extract, CF= crude fiber, NFE= nitrogen free extract, NDF= neutral detergent fiber, ADF = acid detergent fiber and ADL = acid detergent lignin

Chemical evaluation of tested forages:

Chemical composition of the two tested forages was determined for crude protein (CP), crude fiber (CF), ether extract (EE) and ash according to **A.O.A.C. (1995),** while NFE was calculated by difference. Cell wall constituents (NDF, ADF and ADL) were determined according to **Van Soest *et al.* (1991)**. Hemicellulose and cellulose were calculated as the difference between NDF and ADF and between ADF and ADL respectively. Amino acid analyzer (Model 121) was used to determine amino acids content of Berseem and Moringa as described by **Moore *et al.***

(1958). Total phenolic components (TPC) were assayed by Folin-Ciocalteu-reagent 2N (Sigma® -Aldrich, El-Safua Co., Alexandria, Egypt) based on known concentrations of tannic acid as the calibration curve of (Sigma® -Aldrich) according to **Makkar and Becker (1993)**. Condensed tannins (CT) were determined according to **Makkar (2003)**. Mineral extracts of Berseem and Moringa were prepared and analyzed for Ca, K, Na, Cu, Mg, Mn, Fe and Zn after a wet digestion with a mixture of nitric, sulphuric and perchloric acids using an atomic absorption spectrophotometer (Unicam 919). Phosphorus was determined colorimetrically, using molydbovanadate reagent according to **A.O.A.C. (1995)**.

Ruminal degradation kinetics of tested forages:

Three fistulated Barki ewes weighed in average 43.00 kg were used to determine DM and CP degradability of Berseem, Moringa and Berseem + Moringa (50:50). Animals were fed a basal diet of concentrate feed mixture at 0.5 kg/head/ day. Tested forages were oven dried, finally ground and six grams of each were weighed in triplets of each forages for each incubation time. Nylon bags technique was applied to determine degradability of the tested forages according to (**Mehrez and Orskov, 1977**), using polyethylene bags (7x15 cm) with pore size 45 μm. Forage samples were incubated in the rumen of the fistulated sheep for 3, 6, 12, 24, 48 and 72 hrs. All bags were rinsed in tap water for 15 min. until the water become clear, squeezed gently, oven dried and weighed to determine zero-time washing loss (a). Microorganisms attached to the residual sample were eliminated by freezing at 20°C (**Kamel *et al.*, 1995**). Degradation kinetics of DM and CP were estimated by fitting the disappearance values to the equation $P= a+ b (1-e^{-ct})$ as proposed by **Orskov and McDonald (1979)**, where P represents the disappearance after time t. Least-squares estimated of soluble fractions are defined as the rapidly degradable fraction (A), slowly degradable fraction (B) and the rate of degradation (C). The effective degradability (ED) for tested forages were estimated from the equation of **McDonald (1981)** where ED=a+bc/ (c+k), k is the out flow rate.

Nutrients digestibility and nitrogen utilization of experimental rations:

Three digestibility and nitrogen (N) balance trials were conducted on nine Barki uncastrated rams weighed in average 47.5 ± 2.00 kg. Animals were randomly allotted in three equal groups and housed in three separate shaded pens, where each group were fed on one of the three tested rations (R_1, R_2 and R_3) for a preliminary period of four weeks. Animals of each group were then confined individually in nine metabolic cages for 10 days, where the first five days were to get accustom with cages and the second five days for feces and urine collection. Individual amounts of CFM were offered once daily at 8.00 a.m., while green fodders were offered in two equal portions at 8.00 a.m. and 2.00 p.m. Daily amounts of tested rations were calculated according to **NRC (1994)** recommendations for rams. Drinking water was available in buckets at all times. Representative sample of feces from each animal (10% of the whole amount) was oven-dried over- night at 60°C, finally ground and kept in plastic bottle until analysis, while 10% of the daily acidified urine was kept in glass bottles to determine urinary N content. Chemical composition of feeds, feces and urinary N were determined according to **A.O.A.C. (1995)** and cell-wall constituents of feeds and feces were determined according to **Van Soest *et al.* (1991)**. A dietary total digestible nutrients (TDN) was calculated according to the equation of **Cheek *et al.* (1982)**. At the end of digestibility trials, rumen liquor samples were individually collected after three hrs of the morning meal by a rubber stomach tube. Collected rumen liquor was directly tested for pH using Orian 680 digital pH meter, samples were strained through four layers of cheese cloth, while ammonia nitrogen (NH_3-N) was determined by using magnesium oxide (MgO) as described by the **Al-Rabbat *et al.* (1971)**. Total volatile fatty acid (VFA's) concentration was estimated by using steam distillation methods (**Warner, 1964**); molar proportion of VFA's was determined by gas chromatography (**Yang and Varga, 1989**). The microbial nitrogen synthesized (g microbial N/d) in the rumen of sheep fed the experimental rations was calculated using the model equation justified by **Chen *et al.* (1991)** as follows: N supply (g/day) = Pa x 70 (0.83x0.116x1000) where 70 is the N content (mg/mmol) of purines, (Pa mmol/day) is microbial purine absorbed by the animal. The supply of microbial N was then calculated from Pa by assuming that digestibility of microbial purines equals 0.83 and the purine-N: total microbial N ratio equals 0.116.

Feeding experiment of lactating cows:

A feeding experiment was conducted for 90 days, on fifteen multiprous cross bred Friesian cows in their third and fourth lactation season where cows were blocked by weight (555 ± 11.52kg) and previous milk records (10-12 kg / day) in three equal groups. The feeding experiment started 60 days postpartum to allow animals reach normal weight and steady state milk production. Experimental rations based on 60% CFM plus 40%fresh berseem (R_1), or 40% fresh moringa (R_2) and 20% berseem+20% moringa (R3) were offered twice daily at 8.00 a.m. and 2.00 p.m. in two equal portions. Animals of each group (five cows) were housed in three shaded yards and were machinery milked twice daily on feeding times. Daily amounts of tested rations were calculated as recommended by **NRC (2001)**. Drinking water was available at all times. Milk yield was daily recorded for each animal as sum

of morning and after-noon milking. Milk samples (100ml) were collected individually once monthly to determine milk composition changes.

Analytical methods of milk:

Milk fat content was determined according to Gerber's method as described by **Ling (1963)**. Total solids (TS), total protein and ash were determined according to the standard methods of **A.O.A.C. (1995)**. Lactose was determined by the rapid method as described by **John *et al.* (1957)**. Solid not fat (SNF) was calculated by difference. Fat correct milk (4%) was calculated according to **Gaines (1923)** using the following equation: FCM = 0.4 M + 15.0 F, where M = milk yield and F = fat yield.

Blood biochemical constituents:

Blood samples were collected twice from all cows during the last month of the experimental period. Blood samples were withdrawn from the external jugular vein of each animal in heparinized tubes before feeding. Plasma was separated by centrifugation at 4000 rpm for 15-min. Various chemical parameters were calorimetrically determined using commercial kits, following the same steps as described by the manufacturers. Glucose concentration was immediately determined in the whole blood according to **Trinder (1969)**. Total protein (TP) was determined according to **Armstrong and Carr (1964)**; albumin according to **Doumas *et al.* (1971)** and globulin was calculated by subtracting albumin from total protein. Cholesterol was determined according to **Roeschlau *et al.* (1974)**; kidney function was evaluated by measuring blood urea using the colorimetric method of **Henry and Todd (1974)**. Creatinine was measured using the colorimetric method according to **Faulkner and King (1976)**. Liver function was assessed by measuring the activities of aspartate aminotrasferase (AST) and alanine aminotransferase (ALT) according to **Reitman and Frankel (1957).** Enzymatic antioxidants activity in red blood cells was determined for glutathione peroxidase (GSH-Px) according to **Moron *et al.* (1979)**. Catalase was determined according to **Caliborne (1985)**. Superoxide dismutase (SOD) was determined according to **Marklund and Marklund (1974)**.

Statistical analysis:

Collected data of measured parameters were subjected to one way analysis of variance according to **Steel and Torrie (1980)** applying the general linear model procedure of **SAS (2001)**. **Duncan's Multiple Range Test (1955)** was applied to separate significant means.

3. Results and Discussion

Chemical composition and essential amino acids profile of experimental forages:

Data of chemical composition, cell wall constituents, macro and micro-elements content of berseem (*Trifolium alexandrinum*) and *Moringa oleifera* green forages are shown in Table (2). Essential amino acids profile of both forages is presented in Table (3). Moisture content of moringa was lower than berseem (76.42 vs. 81.04%). Dry matter composition showed that moringa forage contained higher crude protein (CP) equals one and half time have that of berseem (18.12vs. 12.84%). Crude fiber (CF) and nitrogen free extract (NFE) were slightly lower in moringa than berseem. Both forages had high ash content being, 9.76% for berseem and 10.14% for moringa. Residual soil particles attached plants during harvesting might be the reason of high ash content. All cell wall constituents were higher in berseem than moringa, however fiber fractions % of moringa in this study were obviously higher than 11.40, 8.49 and 1.8 for neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), respectively as reported by **Moyo *et al.* (2011)** for moringa dry leaves powder. Total polyphenols and condensed tannins were higher in moringa than berseem forage. Polyphenols in this study (2.55%) were lower than 2.7 and 4.3% previously reported by **Gupta *et al.* (1989)** and **Foidl *et al.* (2001)** for moringa leaves. It's worth noting, that at this present concentration simple phenols do not produce any adverse effects when consumed by animals. Meanwhile, these polyphenols have been reported to have multiple beneficial effects that include antioxidant activity, anti-inflammatory action, inhibition of platelets aggregation, antimicrobial and antitumor activities (**Thurber and Fahey, 2009**). Condensed tannins were 1.75% for moringa vs. 0.40% for berseem. Comparable value of condensed tannins being 1.4% was recorded by **Foidl *et al.* (2001)**, for fresh moringa foliage while much higher value (3.12%) was recorded for moringa dry leaves by **Moyo *et al.* (2011)**. However, drying was reported to reduce condensed tannins by 15 to 30% relative to fresh plant (**Vitti *et al.*, 2005**). Macro and micro-elements content were remarkably higher in moringa than berseem except that for sodium and copper which were two times higher in berseem than moringa.

These results were to a great extent in agreement with those optioned by **Moyo *et al.* (2011)** in their comprehensive study on moringa dry leaves cultivated under South Africa eco-system. Calcium had the highest value of 3.69% followed by potassium 1.58% but sodium had the lowest value 0.21%. Among the micro-minerals iron was the highest (397 mg/kg) followed by manganese (80.25 mg/kg), while copper had the lowest value (7.05mg/kg). It is well known that, Ca is required for formation and maintenance of bones and teeth, thus preventing osteoporosis. Interestingly, even iron which is commonly deficient in

many plant-based diets, was found in abundance in moringa leaves. Iron is a necessary component of haemoglobin and myoglobin for oxygen transport and cellular processes of growth and division **(Kozat, 2007).** Iron is also an essential trace element for normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats **(Umar *et al.,* 2007).**Zinc is essential for the synthesis of DNA, RNA, insulin and function and/or structure of several enzymes **(Brisibe *et al.,* 2009).** Zinc is also required for cell reproduction and growth especially sperm cells. In addition, Zn is known for its anti-viral, anti-bacterial, anti-fungal and anti-cancer properties **(Brisibe *et al.,* 2009).** The moringa forage contained copper, which is considered to have strong effects on the immune system **(Anwar *et al.,* 2007).** Copper in combination with zinc could play a role in superoxide dismutase activity and the removal of oxygen free radicals. It is therefore, a key component in the protective mechanism of cellular membranes against superoxide free radicals damage **(Guo *et al.,* 2010).**

Table (2): Chemical composition, cell wall constituents and minerals content of berseem and moringa forages (means ± SD).

Item	Berseem (Ber)	Moringa (Mor)
Moisture, %	81.04 ± 1.16	76.42 ±1.02
DM composition, %:		
Crude protein (CP)	12.84 ± 0.72	18.12 ±0.51
Crude fiber (CF)	26.86 ±1.54	22.73 ±2.18
Ether extract (EE)	1.96 ± 0.31	2.11 ±0.16
Nitrogen free extract (NFE)	48.58 ±2.02	46.90 ±1.25
Ash	9.76 ±1.62	10.14 ±1.97
Cell wall constituents, %:		
Neutral detergent fiber (NDF)	49.86 ±3.65	46.24 ±4.42
Acid detergent fiber (ADF)	37.64 ±3.67	33.85 ±2.38
Acid detergent lignin (ADL)	11.65 ±2.21	9.89 ±1.62
Hemicellulose	12.22 ±1.98	12.39 ±2.11
Cellulose	25.99 ±2.76	23.96 ±3.29
Total poly phenols	1.25 ± 0.11	2.55 ±0.08
Condensed tannins	0.40 ±0.03	1.75 ± 0.27
Macro-elements, %		
Calciaum	1.47 ±0.061	3.69 ±0.044
Phosphorus	0.24 ±0.002	0.33 ±0.005
Magnesium	0.21 ±0.001	0.42 ± 0.003
Sodium	0.49 ± 0.011	0.21 ±0.013
Potassium	0.48 ±0.030	1.58 ±0.021
Micro-elements, ppm		
Iron	126 ±14.03	397 ±37.59
Manganese	34.6 ±3.62	80.2 ±3.45
Zinc	18,5 ±2. 56	44.0 ±4.08
Copper	14.9 ±1.16	7.1 ± 0.14

SD = Standard division.

Table (3): Essential amino acids concentration (g/100g) of berseem and moringa forages (means ± SD).

Item	Berseem (Ber) ± SD	Moringa (Mor) ± SD
Methionine	0.095±0.005	0.12±0.002
Valine	0.67±0.023	0.81±0.035
Leucine	1.04±0.029	1.97±0.015
Phenylalanine	1.11±0.045	1.78±0.011
Threonine	0.61±0.072	0.82±0.069
Tryptophan	0.28±0.006	0.37±0.001
Isolucine	0.48±0.012	0.65±0.006
Lysine	0.63±0.044	1.40±0.067
Tyrosine	ND	1.31±0.024
Histadine	0.074±0.001	1.11 ± 0.008
Total	4.99	10.34

SD = Standard deviation ND = Not detectable

Essential amino acids profile:

Mean values of essential amino acids concentration (g/100g) of dry forages (berseem and moringa) presented in Table (3) illustrate that all values were higher for moringa than berseem. Total essential amino acids content was two times higher for moringa than berseem (10.34 *vs.* 4.99%). The highest value of measured amino acids was recorded for leucine (1.97%) followed by 1.78, 1.40 and 1.31% for phenylalanine, lysine and tyrosine respectively, while the lowest concentration was recorded for methionine in moringa. Tyrosine seemed unavailable in berseem beside its poor contents of histadine (0.074%) and methionine (0.095%). In some previous studies concerning with moringa amino acids content, alanine was noted to has the highest value (3.03%) as mentioned by **Moyo *et al.* (2011)** which was differed than 1.25% reported by **Sainchez-Machado *et al.* (2009)**. The later authors reported leucine had the highest value of 1.75%, which is in agreement with the present findings. However 1.97% of leucine was reported in this study. Methionine in particular is deficient in moringa as in most green leaves. However, methionine as sulphur containing amino acid is very important as a powerful antioxidant help in the detoxification of harmful compounds and protect the body from radiation (**Brisibe *et al.*, 2009 and Moyo *et al.*, 2011**). The present results were in agreement with the findings of **Nuhu (2010)** for moringa concentration of lysine and leucine. The differences in such studies could be attributed to age at harvesting, agro-climatic conditions, soil type, irrigated water quality and handling during harvesting and transportation. Eventhough, moringa could be classified as nutritious forage of good quality protein and rich in most essential amino acids.

Degradation kinetics:

Estimates of ruminal degradation contents (a, b and c) fitted with rates of DM and CP disappearance of tested roughages are presented in Table (4). The results illustrate that washing loss fraction "a', degradable fraction "b" rate of degradation " c" and effective degradability "ED" of DM were lower ($P<0.05$) for berseem than moringa , meanwhile mixing berseem with moringa (1:1) was associated with improving all degradable fractions than for berseem but lower than moringa. The high degradable fractions of moringa DM could be regarded to its high contents of soluble ash and readily fermentable carbohydrates.

Table (4): Degradation kinetics of DM and CP of berseem and moringa forages (means ± SE).

Item	Berseem	Moringa	Berseem + Moringa (1:1)
		DM	
a	31.78 ± 0.18^{b}	34.02 ± 0.34^{a}	32.92 ± 0.28^{ab}
b	34.07 ± 0.85^{c}	45.92 ± 0.62^{a}	40.19 ± 0.44^{b}
c	0.058 ± 0.005^{b}	0.064 ± 0.001^{a}	0.061 ± 0.003^{ab}
EDDM	54.24 ± 0.79^{c}	65.28 ± 0.38^{a}	59.86 ± 0.66^{b}
		CP	
a	25.91 ± 0.21^{a}	22.13 ± 0.18^{c}	24.09 ± 0.17^{b}
b	59.38 ± 1.32^{a}	47.72 ± 0.86^{c}	54.84 ± 0.91^{b}
c	0.071 ± 0.002	0.067 ± 0.001	0.069 ± 0.003
EDCP	67.65 ± 1.84^{a}	55.09 ± 1.43^{c}	62.31 ± 1.60^{b}

$^{a, b, c,}$ Means in the same row with different superscripts are significantly different at ($P< 0.05$).

a= rapidly degradable fraction. b= slowly degradable fraction. c= rate of degradability. EDDM= Effective degradable DM EDCP = Effective degradable CP

Ndemanisho *et al.* (2007) reported that moringa leaves had higher values of ruminal DM degradation kinetics than Leucaena. In the contrast, berseem had the highest CP ($P<0.05$) degradation fractions of a and b and effective degradability "ED" in comparison to moringa either alone or in mixture. **Makkar and Becker (1996)** reported that about 95% of moringa crude protein was found to be available either in the rumen or in the post rumen. The protein potentially digestible in the intestine (PDI) was 47% of the total crude protein of moringa. The PDI is available to the animal for production purposes. They added that PDI values obtained for moringa leaves were much higher than those for various conventional protein supplements like seed meal. High crude protein contents and high PDI values of moringa leaves could suggest that these leaves are good source of protein supplement for high producing cows. **Kleinschmit *et al.* (2007)** cited that proteins that resist degradation in the rumen and pass to the lower tract for digestion " bypass" is necessary for maximizing production of ruminants and high producing dairy animals. They concluded that values of potential and effective degradability and rates of degradation of both DM and CP were affected by diet formulation and levels of fibrous carbohydrates rather than animal species.

Nutrients digestibilities and dietary nitrogen utilization:

Apparent nutrients digestion coefficients of experimental rations are given in Table (5). It's obvious that, R_2 containing (40% moringa) had the highest ($P<0.05$) digestibility values for all nutrients followed by those of R_3 (20% berseem + 20% moringa), while the lowest values were recorded with R_1 containing 40% berseem. The positive effect of moringa on nutrients digestibility could be regarded to its high content of slow degradable protein or essential amino acids needed to enhance rumen microbial activity. Similar assumption was reported by **Poppi and Mclennan (1995)** that feeding moringa forage improved nitrogen supply and corrected N deficiency of low quality diets. Moreover, **Reyes-Sanchez *et al.* (2006_b)** reported that feeding moringa forage had limited effect on rumen fill due to its low NDF content in which feed intake and nutrients digestibility could be improved. As a result of the higher nutrients digestibility associated moringa containing rations, nutritive values expressed as TDN or DCP were ($P<0.05$) higher for R_2 and R_3 than R_1 being respectively, 64.69 and 61.71 *vs.* 58.73 for TDN% and 10.63 and 9.50 *vs.* 8.72 for DCP%.

Table (5) Nutrients digestibility, nutritive value and dietary nitrogen utilization of experimental rations by sheep (means ± SE).

Item	Experimental rations		
	R_1 40% Berseem	R_2 40% Moringa	R_3 20% Berseem +20% Moringa
Nutrients digestibility (%):			
DM	59.97 ± 0.22^c	66.69 ± 0.31^a	64.35 ± 0.27^b
OM	62.57 ± 0.17^c	68.79 ± 0.25^a	65.78 ± 0.33^b
CP	59.12 ± 0.43^c	62.93 ± 0.29^a	60.14 ± 0.09^b
CF	55.98 ± 0.18^c	59.11 ± 0.11^a	57.25 ± 0.21^b
EE	61.53 ± 0.32^b	73.84 ± 0.48^a	64.54 ± 0.39^b
NFE	65.68 ± 0.37^c	73.31 ± 0.29^a	70.15 ± 0.31^b
Nutritive value (%):			
Total digestible nutrients (TDN)	58.73 ± 0.54^c	64.69 ± 0.17^a	61.71 ± 0.33^b
Digestible crude protein (DCP)	8.72 ± 0.36^c	10.63 ± 0.26^a	9.50 ± 0.32^b
Nitrogen utilization, g			
Nitrogen intake (NI)	24.01 ± 0.62^c	28.24 ± 0.18^a	25.94 ± 0.33^b
Fecal nitrogen (FN)	9.82 ± 0.42^b	10.33 ± 0.31^a	10.33 ± 0.45^a
Digestible nitrogen (DN)	14.19 ± 0.71^c	17.91 ± 0.09^a	15.61 ± 0.12^b
Urinary nitrogen (UN)	8.99 ± 0.51^b	9.62 ± 0.46^a	9.22 ± 0.66^{ab}
Total nitrogen out-put	18.81 ± 0.23^b	19.95 ± 0.62^a	19.55 ± 0.51^a
Apparent retained N (ARN)	5.20 ± 0.19^c	8.29 ± 0.03^a	6.39 ± 0.09^b
ARN of NI, %	21.66 ± 0.57^c	29.35 ± 0.14^a	24.63 ± 0.17^b
ARN of DN. %	36.64 ± 1.77^c	46.29 ± 0.29^a	40.94 ± 0.36^b

$^{a, b \text{ and } c}$: means with different superscripts in the same row are significantly different at ($p < 0.05$).

The present results are in good agreement with those reported by **Newton *et al.* (2010)**, **Mendieta-Araica *et al.* (2013)** and **Nouman *et al.* (2013)**. They also reported that moringa forage is rich in most nutrients as its addition to low quality diets is useful to increase their dry matter intake and nutrients digestibility.

Results of N-balance as well showed remarkable ($P < 0.05$) increase of apparent retained nitrogen (ARN) calculated relative to N-intake or digestible N with moringa containing rations. Values of ARN% of N-intake were 29.35, 24.63 and 21.66, and relative to digestible N were 46.29, 40.94 and 36.64 for R_2 (40% moringa), R_3 (20% berseem + 20% moringa) and R_1 (40% berseem), respectively. The results indicate that feeding moringa forage in partial or complete substitution of berseem fodder improved dietary N utilization (ARN of N-intake %) by nearly 35% with R_2 (40% moringa) and 14% with R_3 (20% berseem + 20% moringa) in comparison to R_1 (40% berseem). These results confirmed the previous findings of **Mendieta-Aracia *et al.* (2013)** and **Nouman *et al.* (2013)** that moringa leaves had good quality protein rich of essential amino acids which can enhance dietary N utilization and improve animal productivity.

Rumen fermentation:

Rumen fermentation activity of animals fed experimental rations is given in Table (6). Rumen liquor pH values and NH_3-N concentration were lower ($P<0.05$) with 40% moringa ration (R_2) than those containing 40% berseem (R_1) or 20% berseem (R_3), while total VFAs concentration was remarkably higher

by nearly 31% and 32% for R_2 and R_3 respectively, than R_1 containing 40% berseem. In similar trend, microbial-N yield was greater with the two moringa rations (R_2 and R_3) than that of 40% berseem containing ration.

Molar proportion % of propionic and butyric acids was insignificantly different among groups. However, moringa containing rations showed higher acetic acid content than that of R_1 (40% berseem ration). These results are matching the results of degradation kinetics (Table 4) that moringa had higher DM effective degradability but lower soluble and degradable CP than berseem forage. Moreover, the obvious high microbial-N yield and apparent dietary-N utilization (Table 5) might indicate that moringa containing rations improved the synchrony between dietary energy and protein which was resulted in lower ruminal ammonia-N and higher VFA's than berseem. The present results confirmed the previous results of **Hoffmann *et al.* (2003)** who stated that, the low ruminal NH_3-N associated moringa supplementation is attributed to its low protein degradability. **Soliva *et al.* (2005)** concluded that moringa leaves are not suggested as a source of rumen protected protein. They proposed that it promotes rumen microbial protein synthesis due to its substantial contents of readily fermentable N and energy. They concluded that it still has to be shown whether or not this protein is arriving at the duodenum of the ruminant and in how far these feeds are competitive to the more common protein sources in highly productive growing or milk producing ruminants. However, **Alexander *et al.* (2008)** found that NH_3-N concentration was decreased when a medium of white clover hay was incubated with moringa leaves extract. Some other studies mentioned that, the relatively high contents of tannins and saponins which are naturely occurring in moringa leaves could affect ruminal proteolytic activity resulted in lower ruminal ammonia-N (**Oliveira *et al.*, 1999, Sliwinski *et al.*, 2002 and Soliva *et al.*, 2005**). The later assumption may not hold true since VFA's concentration and microbial yield in the present study were higher with moringa rations, however, moringa contained higher tannins and polyphenols than berseem (see Table 2).

Table (6): Rumen fermentation parameters of sheep fed experimental rations (means ± SE).

Item	Experimental rations		
	R_1 40% Berseem	**R_2 40% Moringa**	**R_3 20% Berseem + 20% Moringa**
pH	6.52 ± 0.12[a]	6.44 ± 0.09[b]	6.49 ± 0.11[ab]
NH_3-N concentration (mg/100 m/R.L)	15.54 ± 0.17[a]	12.08 ± 0.11[c]	13.67 ± 0.08[b]
VFA concentration (meq/100 m/R.L)	11.85 ± 0.22[b]	15.53 ± 0.29[a]	14.59 ± 0.24[ab]
Molar proportion %			
Acetic acid	58.10 ± 0.85[b]	62.09 ± 0.78[a]	60.70 ± 1.09[ab]
Propionic acid	25.76 ± 0.47	25.08 ± 0.61	25.27 ± 0.74
Butyric acid	10.08 ± 0.29	8.85 ± 0.21	9.07 ± 0.39
Acetic: Propionic ratio	2.26 ± 0.02[b]	2.48 ± 0.04[a]	2.40 ± 0.05[a]
Microbial N yield (g/d)	13.91 ± 0.08[c]	16.25 ± 0.10[a]	15.45 ± 0.11[b]

[a,b and c] : means in the same row with different superscripts are significantly different at (P< 0.05).

Lactation performance:

Milk yield and composition of cows fed experimental rations are presented in Table (7) and lactation curve of cows in each group is shown in Fig (1). Fresh milk and 4% FCM yields were higher (P< 0.05) with rations contained either 40% or 20% moringa (R_2 and R_3) than those fed 40% berseem ration. Lactation curve of experimental groups (Fig. 1) illustrates that the positive effect of feeding moringa rations (R_2 and R_3) on milk production was realized within the first two weeks of feeding and the improvement extended up to end of the feeding period. The improvement of milk production as 4% FCM was 25.4% for R_2 and 16.0% for R_3 groups compared to that of group R_1 (40% berseem). Moreover, milk constituents including total solids, solids not fat, fat, protein and ash were (P< 0.05) increased with feeding moringa forage, however the effect of forage type on milk constituents was more pronounced with the complete substitution of berseem with moringa (R_2). Meanwhile, differences of milk lactose content did not attain significancy among groups. Energy density (Kcal/kg milk) was obviously higher for milk of cows fed 40% or 20% moringa than that of cows fed R_1, being respectively, 719.7 and 683.9 vs. 637.0 Kcal.

These results could be attributed to the influence of moringa leaves on ruminal fermentation enhancement, investigated from higher TVFA's concentration and microbial-N yield associated moringa rations (see Table 6). Similar assumption was reported by **Sarwatt *et al.* (2004)** who stated that the small amounts of moringa leaves improved the rumen environment which was implied on better feed utilization and milk production. In agreement of the

present results, **Reyes Sanchez *et al.* (2006_b)** found that daily milk production was significantly (P<0.05) higher for cows fed *M. oliefera* supplement than those fed *B. brizan* hay only. They added that the improvement of milk production was associated with an increase of fat and protein yields. **Mendieta-Aracia (2011)** reported that *M. oliefera* supplements did not affect milk organoleptic characteristics including taste, smell or color. The good influence of moringa leaves on milk production and composition was also reported by **Basitan and Emma (2013)**, who found that cows fed moringa supplemented rations had higher (P< 0.05) milk and fat yields than cows fed free moringa ration.

Blood biochemical constituents:

Data of blood analysis given in Table (8) illustrate that blood glucose, total protein, albumin and globulin were higher (P<0.05) for cows fed moringa rations (R_2 and R_3) than those fed berseem (R_1).

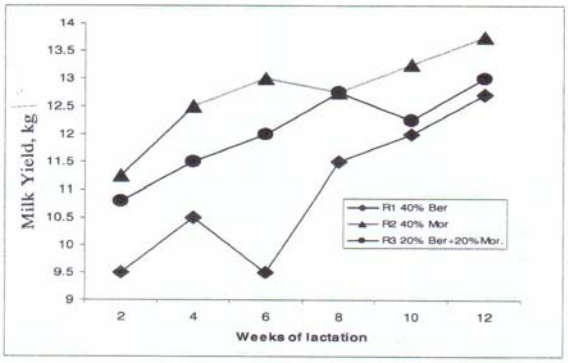

Fig. (1): Lactation curve of cows fed experimental rations.

Table (7): Milk yield and milk constituents of cows fed experimental rations (means ± SE).

Item	Experimental rations		
	R1 40% Berseem	**R2 40% Moringa**	**R3 20% Berseem + 20% Moringa**
No. of animals	**5**	**5**	**5**
Lactation period,	---------- **90 days** ----------		
Milk production, Kg/day			
Milk yield	10.95 ± 0.66^b	12.75 ± 0.42^a	12.05 ± 0.58^a
4% FCM yield	9.33 ± 0.12^c	11.7 ± 0.16^a	10.82 ± 0.11^b
Milk fat	$0.33 \pm 0.0b^c$	0.44 ± 0.03^a	0.40 ± 0.02^b
Milk protein	0.32 ± 0.04^b	0.42 ± 0.06^a	0.39 ± 0.05^a
Milk energy, kcal*	637.0 ± 5.17^c	719.7 ± 6.62^a	683.9 ± 1.58^b
Milk constituents, %			
Total solids (TS)	11.46 ± 0.35^c	12.73 ± 0.27^a	12.15 ± 0.21^b
Solids not fat (SNF)	8.44 ± 0.25^b	9.24 ± 0.19^a	8.83 ± 0.26^a
Fat	3.02 ± 0.11^c	3.49 ± 0.25^a	3.32 ± 0.07^b
Protein	2.92 ± 0.10^b	3.33 ± 0.15^a	3.26 ± 0.12^a
Lactose	4.74 ± 0.16	4.99 ± 0.12	4.73 ± 0.09
Ash	0.78 ± 0.03^b	0.92 ± 0.05^a	0.84 ± 0.03^{ab}

a, b and c means with different superscripts in the same row are significantly different at (p< 0.05) .

** Calculated according to the equation suggested by **Mc Donald et al. (1978)**: Kcal/ kg milk = 92.25x fat% + 49.15x SNF%-56.4.*

Table (8): Blood biochemical constituents of lactating cows fed experimental rations (means ± SE).

Item	Experimental groups		
	R_1 40% Berseem	**R_2 40% Moringa**	**R_3 20% Berseem +20% Moringa**
Glucose, mg/dl	95.56 ± 0.58^b	109.83 ± 0.49^a	101.95 ± 0.41^{ab}
Cholesterol, mg/dl	121.44 ± 0.43^a	94.76 ± 0.22^c	97.88 ± 0.23^b
Total protein, g/dl	7.53 ± 0.16^b	8.78 ± 0.28^a	7.89 ± 0.22^b
Albumin, (A) g/dl	4.20 ± 0.11^b	4.95 ± 0.24^a	4.42 ± 0.16^a
Globulin, (G) g/dl	3.33 ± 0.10^b	3.83 ± 0.11^a	3.47 ± 0.14^b
A/G ratio	1.26 ± 0.02	1.29 ± 0.05^a	1.27 ± 0.03^{ab}
Urea, mg/dl	46.45 ± 0.17^a	38.44 ± 0.27^b	42.35 ± 0.14^{ab}
Creatinine, mg/dl	0.99 ± 0.10	0.91 ± 0.14	0.91 ± 0.12
AST, U/L	35.77 ± 0.43	34.28 ± 0.52	35.65 ± 0.32
ALT, U/L	19.43 ± 0.16	18.66 ± 0.22	19.32 ± 0.41

a, b and c : means in the same row with different superscript are significantly different at (P < 0.05).

Moreover, blood cholesterol and urea were decreased with moringa rations, however the effect was more pronounced with 40% moringa ration. But creatinine, aspartate (AST) and alanine (ALT) transaminases were comparable among groups. In other words, feeding moringa up to 40% of the whole daily ration did not badly affects liver or kidney functions.

The present values of the three experimental groups were within the normal range of cows (**Stanek *et al.*, 1992**). However, the significant high blood glucose level with moringa feeding might support the assumption that, feeding moringa forage could help in bypassing some soluble carbohydrates to be absorbed as glucose which helps in increasing the metabolizable energy intake. In this concern, **Annison *et al.* (2002)** found a linear relationship between glucose entry rate and metabolizable energy intake. On the other hand, the significantly lower cholesterol level associated feeding moringa might be related to the higher phytonutrients content of moringa than other common forages. **Astuti *et al.* (2011)** reported that rations contained *M. oleifera* with certain amount of saponin had good effect on animal health as expressed in low serum cholesterol and normal essential fatty acids concentration. The lower blood urea level associated feeding moringa forage was expected from the higher dietary N utilization of rations containing moringa than that contained berseem (see Table 5). **Hoffmann *et al.* (2003)** assumed that, the high utilization of moringa nitrogen could be regarded to its cationic protein and rumen microbes interaction that making them available in the small intestine in an intact form. Anyway, there is a need for more studies concerning with energy and protein utilization of fresh or dry moringa leaves in the feeding practices of dairy cattle.

Blood antioxidant enzymes:

Enzymatic antioxidants activity of cows fed experimental rations presented in Table (9) showed that glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) were significantly ($P<0.05$) increased with rations containing moringa, however the highest values were recorded for cows fed 40% moringa forage (R_2). Catalase enzyme activity was highly remarkable with feeding 40% moringa in comparison to 40% berseem ration (185.87 *vs.* 99.76 µ mol/g protein). Antioxidant enzymes mainly SOD and CAT are the first line defensive against free radicals which cause oxidative damage in animal tissues. Catalase (CAT) is one of the most important intracellular enzymes in the detoxification of the oxidant hydrogen peroxide. The activity of CAT and SOD enzymes is inhibited with high level of toxic metabolites (**Visavadiya and Narasimhachary, 2008**). Glutathione peroxidase (GSH-Px) is the most powerful antioxidant enzyme protects cellular proteins against reactive oxygen species (ROS) in the body (**Arivazhagan *et al.*, 2000**).

Table (9): Enzymatic antioxidants activity in blood of lactating cows fed experimental rations (mean ± SE).

Item	Experimental groups		
	R_1 40% Berseem	**R_2 40% Moringa**	**R_3 20% Berseem + 20% Moringa**
GSH-Px, µ g/g protein	7.66 ± 0.45[c]	9.94 ± 0.15[a]	8.57 ± 0.36[b]
CAT, µ mol/g protein	99.76 ± 3.65[c]	185.87 ± 2.94[a]	121.65 ± 1.78[b]
SOD, IU/g protein	12.37 ± 0.54[c]	15.88 ± 0.22[a]	13.96 ± 0.16[b]

[a, b and c] Means in the same row with different superscripts are significantly different at ($P< 0.05$).

The present results revealed that feeding moringa rations had potential effect on increasing internal antioxidant enzymes of experimental cows. It was reported that *M. oleifera* leaves contained flavonoids such as Kaempferol, rhamnetin, isoquercitrin and Kaempferitrin. These polyphenolic compounds could significantly contribute in scavenging free radicals or act as free radical terminator (**Iqbal and Bhanger, 2006; Pourmorad *et al.*, 2006; Khalafalla *et al.*, 2010 and Satish *et al.*, 2013**). Similar conclusions were reported in some earlier studies that, flavonoids could carry out antioxidant action through scavenging or chelating process (**Middleton *et al.*, 2000**). Methanolic extract of moringa leaves was found to have antioxidant properties and there was a strong relationship between polyphenolic compounds and antioxidant activity (**Siddhuraju and Becker, 2003, Odukoya *et al.*, 2005**). In more recent studies, **Asokkumar *et al.* (2008)** stated that moringa had potential antioxidant effect by quenching free radicals. **Oyedemi *et al.* (2010)** stated that, moringa can reduce reactive free radicals that might lessen oxidative damage in the tissues through hydrogen peroxide decomposition. While, **Choi *et al.* (2010)** found that the antioxidant potential of plants stimulate GSH activity in rats. The mode of action of plant antioxidant compounds was explored by **Venkatesan *et al.* (2012)**, who stated that external antioxidants might enhance phagocytic activity and increase stimulation of immune and antioxidant activity. So that, *M. oleifera* is a rich source of antioxidant compounds i.e.; flavonoids, Vit A, B, C and E beside some other trace minerals (Zn,

Cu, Se and Fe) which could eliminate the free radical harmful effect, and in turn considered an added value to its nutritional quality in feeding ruminants.

Feed conversion and economic efficiency:

Feed intake, milk production, feed conversion and economic evaluation given in Table (10) show that, daily DM intake was nearly comparable among groups being; 11.61, 11.95 and 11.73 kg/h for R_1, R_2 and R_3, respectively, indicated that both forages (berseem and moringa) had similar degree of palatability. The significant difference between groups in terms of TDN intake is regarded to the higher nutrients digestibilites of moringa containing rations R_2 (40% moringa) and R_3 (20% moringa) than R_1 (40% berseem). Feed conversion (Kg DM/kg 4% FCM) was improved ($P< 0.05$) by nearly 18% and 13% with 40% and 20% moringa rations in comparison to that with R_1 (40% berseem).

Table (10): Feed intake, feed conversion and economic evaluation of milk yield of cows fed experimental rations (means ± SE).

Item	Experimental groups		
	R_1 40% Berseem	**R_2 40% Moringa**	**R_3 20% Berseem + 20% Moringa**
Feed intake, kg/h/day:			
Dry matter (DMI)	11.61 ± 0.09	11.95 ± 0.10	11.73 ± 0.04
Total digestible nutrients intake (TDNI)	6.82 ± 0.12^b	7.73 ± 0.14^a	7.23 ± 0.19^{ab}
Yield of 4% FCM, kg	9.33 ± 0.12^c	11.70 ± 0.16^a	10.82 ± 0.11^b
Feed conversion (kg/kg 4% FCM):			
DMI	1.24 ± 0.08^a	1.02 ± 0.09^b	1.08 ± 0.04^b
TDNI	0.73 ± 0.02^a	0.66 ± 0.02^b	0.67 ± 0.01^b
Economic evaluation *			
Daily feeding cost, L.E.	26.38	25.41	25.86
Price of the daily fresh milk yield, L.E.	32.85	38.25	36.15
Revenue of feeding cost, L.E.	6.47	12.84	10.29
Relative economic efficiency, %**	100	198	159

$^{a,b \text{ and } c}$: means with different superscripts in the same row are significantly different at ($P< 0.05$).

* Based on local prices of year 2013: Berseem clover = 230 L.E/ton. Moringa forage = 220 L.E. / ton, CFM = 2600 L.E. / ton. Average selling price of kg fresh milk = 3.00 L.E.

** Assuming that the relative economic efficiency of the 40% Berseem ration equals 100% .

Rate of exchange for one US$ = 7.00 L.E.

Aregheore (2002) found that feeding *M. oleifera* at 20 and 50% of the total daily forage intake by goats was associated with better dietary protein utilization and feed conversion than those fed Batilhi grass. Daily revenue of feeding cost (L.E./h) was 12.84 and 10.29 with R_2 and R_3 respectively, *vs.* 6.47 for R_1 (40% berseem ration). Economic efficiency relative to revenue of R_1 (assuming revenue of R_1 equals 100%) was 198 and 159% for groups fed R_2 (40% moringa) and R_3 (20% moringa). In this context, **Adegum and Aye (2013)** reported that the cost of production was reduced when *M. oleifera* leaf meal replaced cotton seed meal in rations of dairy cows, which in turn increase profit and improve the living standard of farmers.

Conclusion:

From the results of this study, it's fair to conclude that introducing *Moringa oleifera* green forage in partial or complete replacement of berseem (*Trifolium alexandrinum*) fodder is highly recommended to improve lactation performance, health status and economic revenue of local dairy Friesian cows.

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9/26/2014