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# Yield and composition of milk and detection of plasma ghrelin and IGF – 1 in dairy buffalo fed with *Moringa oleifera* leaf meal (MoLM) supplement

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**Abstract.** The study investigated the nutritive effects of *Moringa oleifera* leaf meal (MoLM) in terms of milk yield and composition and detection of putative plasma ghrelin and IGF-1 in dairy buffalo. A total of eight (8) lactating dairy buffalo in mid lactation stage were randomly allotted to two treatments (T1- Control and T2-30% MoLM + 70% Concentrate). Milk samples were analyzed using MilkoScan FT-1 milk analyzer. Data on the milk yield and milk composition were analyzed using factorial analysis in CRD by means of Statistix 10. Blood samples were collected before the during the feeding trial to test the presence of ghrelin and IGF-1 concentration using SDS-PAGE. In terms of milk yield and composition, no significant differences were noted between treatments but significant difference was noted in terms of milk collection on milk protein, lactose, TS and SNF. Both hormones (ghrelin and IGF-1) were detected in the blood of experimental animals before and after the feeding trial. This study demonstrated that MoLM can be a good alternative for commercial feed for dairy buffaloes.

## 1. Introduction

One way to increase milk production is through diet modification using protein-rich supplement like *Moringa oleifera* leaf meal (MoLM) [1]. *Moringa* leaves are also rich in carotene, iron, ascorbic acid [2]. The leaves of *M. oleifera* are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper [3]. This plant contains phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugar present along with anti-cancerous agents like glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate [4].

In cows and does, its galactagogue properties have already been proven but information on the feeding value of MoLM in relation to buffalo production specifically in milk production are few. In relation to this, the study was focused on investigating the effects of MoLM and interval of milk collection in terms of milk yield and milk composition, and detection of putative plasma ghrelin and IGF-1. Ghrelin is small synthetic molecules called growth-hormone secretagogues (GHSs) that



stimulate the release of growth hormone (GH) from the pituitary [3]. On the otherhand, Insulin-like Growth Factor – 1 (IGF-1) is the mediator of the anabolic and mitogenic activity of GH [5]. Both the plasma ghrelin and IGF-1 are involved in partitioning the energy and nutrients towards milk production in dairy buffalo.

Results from this study aimed to produce new knowledge that will help dairy buffalo raisers improve their milk production and consequently increase their income.

## 2. Materials and Methods

A total of eight (8) lactating buffaloes weighing from 481 to 534 kilograms, five (5) parities and at the same lactation stage (mid lactation) were randomly allotted to two treatments. Each treatment was replicated four times with one animal per replicate. The dairy buffaloes were confined in individual pens and acclimatized for two weeks. The animals were assigned to the following treatments: T1 – Control (1.25% urea + 98.75% concentrate and T2 - 30% MoLM + 70% concentrate).

The dairy animals were given 70% fresh forages and 30% concentrates. MoLM was sourced out from different barangays in the municipalities of Ilagan, Tumauini, Delfin Albano, Cabagan, Sta Maria and Sto. Tomas Isabela. Green moringa leaves were harvested and dried under the sun for 72 hours.

Sun dried moringa leaves were ground and mixed in the concentrate diet of the animal at 30% level of the total concentrates.

The concentrates offered to the experimental animals was formulated by Gromax Incorporated using cereals, soybean meal, copra meal, molasses, rice bran, pollard, distillers dried grains soluble, limestone, biofos, vitamins, minerals and mold inhibitors as ingredients. The crude protein CP, ether extract (EE) and calcium (Ca) content of the concentrates was 16.00, 7.00, and 0.90% respectively. The proximate composition of the concentrates diet of the experimental animal is shown in Table 1.

**Table 1.** Proximate composition (%) of the different concentrates offered to control and treated animals (dry matter basis).

	Treatment 1	Treatment 2
<i>Proximate Analysis</i>		
Crude Protein (CP)	18.59	18.67
Crude Fiber (CF)	7.64	7.61
Ether Extract (EE)	6.81	7.39
Nitrogen Free Extract (NFE)	54.68	54.18
Ash	7.13	7.91
<i>Mineral Analysis</i>		
Calcium (Ca)	1.24	1.71
Phosphorus (P)	0.68	0.60
Gross Energy (kcal/kg)	3983.67	3785.67

The experimental animals were fed with MoLM mixed concentrates twice a day (early morning and late afternoon) and forage for 63 days. The amount of forage and concentrates given to the experimental animals was based on 5% dry matter requirements of the animals.

Prior to the administration of the supplements, the weights of the experimental animals were taken. After acclimatization, individual milk yield was collected and served as the initial milk yield. Milk was collected twice a day at 4:00 a.m. and 3:00 p.m. and recorded daily.

Concentrate diets of the lactating buffalo were analyzed by proximate analysis using standard AOAC methods 2000 [5].

Individual samples of milk were analyzed using MilkoScan FT-1 milk analyzer which analyses fat, protein, lactose, total solids, solid non fat of milk.

Before and after the administration of moringa in buffaloes' diet, blood samples were collected. Collection of blood was done in the morning (3:30 a.m) at a two-week interval. Blood plasma was harvested after centrifugation and used in detecting the presence of putative ghrelin and IGF-1 hormones using Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis (PAGE).

Data on milk yield and milk composition were analyzed using a Complete Randomized Design (CRD). Factor 1 was the different concentrates offered while factor 2 was the interval of milk sampling.

### 3. Results and Discussion

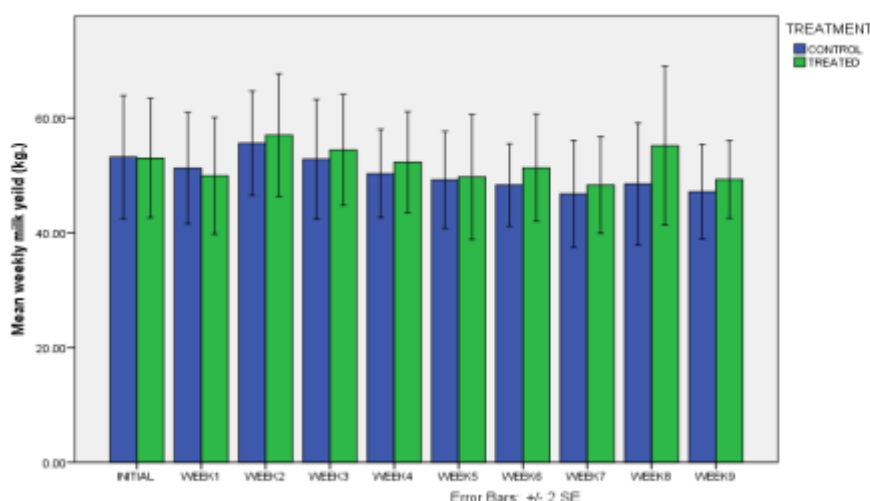
#### 3.1. Milk yield

For nine (9) weeks of feeding trials, both Treatments 1 and 2 obtained the highest milk yield in the 2<sup>nd</sup> week while the lowest was in the 7<sup>th</sup> week (Table 2).

**Table 2.** Average milk yield of lactating buffaloes fed with control and supplemented with 30% MoLM concentrates.

Weekly Collection	Treatment 1	Treatment 2
Initial	53.20	53.03
Week 1	51.28	49.95
Week 2	55.60	57.03
Week 3	52.83	54.45
Week 4	50.33	52.33
Week 5	49.25	49.73
Week 6	48.33	51.33
Week 7	46.78	48.33
Week 8	48.53	50.20
Week 9	47.18	49.33
MEAN $\pm$ SEM	50.33 $\pm$ 4.61	51.57 $\pm$ 5.03

In Treatment 2, an increase in milk yield of about of 0.48 kgs to 3.00 kgs per day was recorded.



**Figure 1.** Trend of milk yield of lactating buffaloes for 9 weeks feeding.

See-saw pattern was the trend of milk yield obtained in the study (Figure 1). Before feeding the experimental animals with test concentrates, the milk yield was high. However, a significant decrease

in the milk yield was noticed after the first administration of the test concentrates. On the 3<sup>rd</sup> week of the administration, the milk yield increased again to a certain level and then declined gradually during the succeeding weeks. Abrupt drop on milk yield was again noted on the 7<sup>th</sup> week but increased again in the succeeding weeks of collection.

Decreased milk yield on the first week was due to the adjustment of the animals on the changes to the feeds offered. Abrupt drop in milk yield noted on the 7<sup>th</sup> week could be due to climatic stress brought about by the typhoon Glenda. It affected the feed intake of both control and treated animals. According to Mauser et al [7] climatic changes (temperature and humidity) greatly affect milk production in dairy animals. During the succeeding weeks of feeding, the animals were observed to have gradually recovered. No significant difference was noted between the two treatments and on different milk collections.

### 3.2. Milk composition

Milk collected before and after the administration of the two (2) treatments were examined. The percentage of EE, CP, lactose, TS and SNF were analyzed using milk analyzer. The result of the analyses is shown on Table 3.

**Table 3.** Milk fat (%) content of lactating buffaloes fed with control and supplemented with 30% MoLM concentrates.

Weekly Collection	Treatment 1	Treatment 2
Initial	8.70	7.82
1 <sup>st</sup> Collection	9.21	8.58
2 <sup>nd</sup> Collection	7.84	8.90
3 <sup>rd</sup> Collection	8.77	8.88
4 <sup>th</sup> Collection	8.68	9.44
MEAN $\pm$ SEM	8.64 $\pm$ 1.05	8.72 $\pm$ 0.58

The percentage milk fat in Treatment 1 ranged from 7.88 to 9.21% while in Treatment 2, it ranged from 8.58 to 9.44%. Low milk fat content ranging (6.40 - 7.70%) was reported Caroian et al. [8].

The result of this study on the fat content of buffaloes' milk was in accordance with the findings of Khan et al. [6]. Slightly higher fat percentage value was obtained in this study compared to the values recorded this is due to the fat content of the feeds offered (with MoLM) to the experimental animals is higher but statistically, no significant difference was noted between the two treatments and milk collection.

According to Caroian et al. [8] fat content varies depending on the stage of lactation and milk production volume. The fat content level tends to decrease from 1<sup>st</sup> to 4<sup>th</sup> lactation and it gradually increases until it reaches the maximum value. Slight decrease of milk fat will follow on the 8<sup>th</sup> lactation. The percentage fat of buffaloes milk increases steadily from 5.5 to 7.5% in the first and 10<sup>th</sup> month of lactation.

Protein percentage showed no significant difference between the two treatments (Table 4). However, there was a significant difference of milk protein percentage between the initial milk collection and the succeeding milk collections.

Studies conducted by Han et al.[9] and Caroian et al.[8] showed protein percentage ranging from 4.00- 5.00%. The protein content obtained in this study was in accordance with the findings of Imran et al.[10]. Compared to the values recorded by other researchers, higher protein percentage was recorded in this study. Increasing protein content of the milk extracted from both treatments maybe due to high protein content of the feeds offered to the experimental animals.

**Table 4.** Milk protein (%) content of lactating buffaloes fed with control and supplemented with 30% MoLM concentrates.

Weekly Collection <sup>1</sup>	Treatment 1	Treatment 2
Initial <sup>c</sup>	3.81	4.15
1 <sup>st</sup> Collection <sup>b</sup>	4.94	4.91
2 <sup>nd</sup> Collection <sup>a</sup>	5.65	5.63
3 <sup>rd</sup> Collection <sup>a</sup>	5.77	5.42
4 <sup>th</sup> Collection <sup>a</sup>	5.48	5.51
MEAN $\pm$ SEM	5.13 $\pm$ 0.29	5.12 $\pm$ 0.63

<sup>1</sup>Superscript with different letters are significant ( $P \leq 0.05$ )

The lactose content in Treatment 1 ranged from 5.12 to 6.44% while in Treatment 2 it ranged from 4.77 to 6.46% (Table 5).

**Table 5.** Milk lactose (%) content of lactating buffaloes fed with control and supplemented with 30% MoLM concentrates.

Weekly Collection <sup>1</sup>	Treatment 1	Treatment 2
Initial <sup>b</sup>	4.86	4.60
1 <sup>st</sup> Collection <sup>b</sup>	5.12	4.77
2 <sup>nd</sup> Collection <sup>a</sup>	6.16	6.43
3 <sup>rd</sup> Collection <sup>a</sup>	6.44	6.46
4 <sup>th</sup> Collection <sup>a</sup>	6.33	6.42
MEAN $\pm$ SEM	5.78 $\pm$ 0.35	5.74 $\pm$ 0.20

<sup>1</sup>Superscript with different letters are significant ( $P \leq 0.05$ )

On the other hand, Han et al. [9] and Caroian et al. [8] obtained 4.60 to 5.41% milk lactose. Significant difference was noted in milk collection where 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> collections are comparable to the initial and 1<sup>st</sup> milk collections. The result of the study indicates that prolong feeding of the two treatments will gradually increase the amount of lactose in milk.

The percent milk total solids of the two Treatments ranged from 19.95 to 24.02% and 19.21 to 24.07%. The analysis showed no significant difference. Comparable results were observed on different times of milk collections (Table 6).

**Table 6.** Total solids (%) content of lactating buffaloes fed with control and supplemented with 30% MoLM concentrates.

Weekly Collection <sup>1</sup>	Treatment 1	Treatment 2
Initial <sup>c</sup>	18.81	17.86
1 <sup>st</sup> Collection <sup>bc</sup>	19.95	19.21
2 <sup>nd</sup> Collection <sup>ab</sup>	22.93	22.10
3 <sup>rd</sup> Collection <sup>a</sup>	23.34	24.44
4 <sup>th</sup> Collection <sup>a</sup>	24.02	24.07
MEAN $\pm$ SEM	21.81 $\pm$ 1.68	21.54 $\pm$ 1.14

<sup>1</sup>Superscript with different letters are significant ( $P \leq 0.05$ )

The TS ranges from 16.99 to 20.18% was recorded by Braun et al.[11]. The milk TS percentage obtained by Han et al. [9] ranged from 16.70 to 18.18%. Higher values of TS were obtained in this experiment than the values obtained from different studies due to the nutrients present in moringa which contributes in the milk composition.

Meanwhile, the percentages of SNF were similar in both treatments (Table 7). Higher SNF value was recorded in this experiment than to the data obtained by Ahmad et al. [12].

In terms of milk collections, 2<sup>nd</sup> – 4<sup>th</sup> week values were higher ( $P < 0.05$ ) than those obtained from initial to 1<sup>st</sup> week.

**Table 7.** Solid non fat (%) content of lactating buffaloes fed with control and supplemented with 30% MoLM concentrates.

Weekly Collection <sup>1</sup>	Treatment 1	Treatment 2
Initial <sup>b</sup>	9.49	9.52
1 <sup>st</sup> Collection <sup>b</sup>	10.74	10.47
2 <sup>nd</sup> Collection <sup>a</sup>	10.74	10.47
3 <sup>rd</sup> Collection <sup>a</sup>	13.24	13.22
4 <sup>th</sup> Collection <sup>a</sup>	14.65	14.70
MEAN $\pm$ SEM	11.77 $\pm$ 1.26	11.68 $\pm$ 0.69

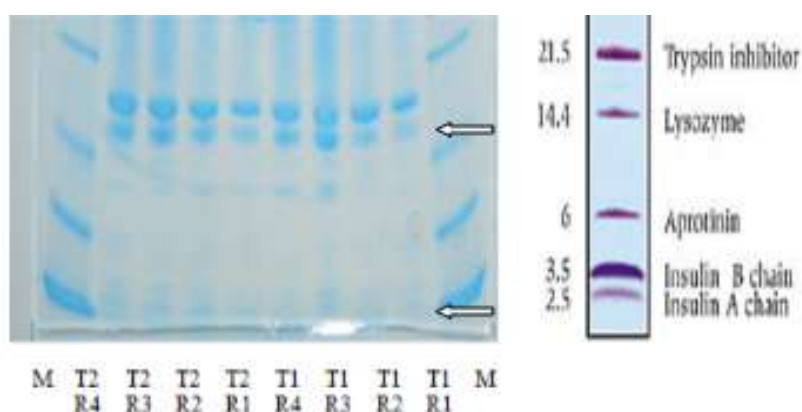
<sup>1</sup>Superscript with different letters are significant ( $P \leq 0.05$ )

No significant difference in milk composition was observed in cows when fed with MoLM, SBM and “Iso” concentrates [14]. The same findings was observed by Sanchez et al. [1] in using 2 or 3 kg of Moringa in cows.

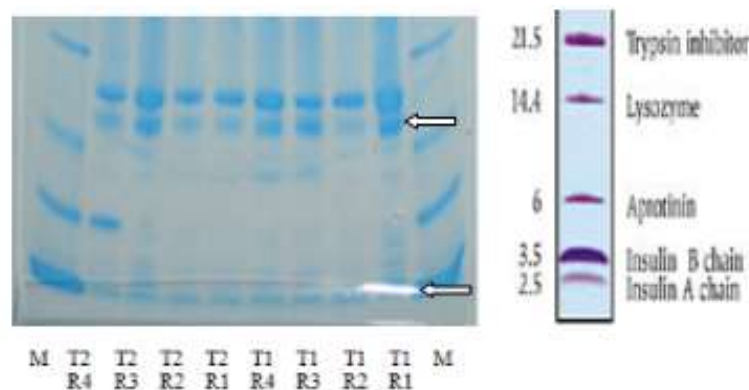
Prolong feeding of MoLM resulted to increase in SNF content of buffalo milk this maybe attributed by potent nutrients present in MoLM that are used during the formation of milk.

### 3.3. Putative ghrelin and IGF-1

The images of the gels after electrophoresis are shown Figures 2 and 3. In the gel, there was noticeable band between lysozyme (14.4kda) and aprotinin (6kda). This band is a possible indication of the presence of IGF-1(7.649kda) hormone. Ghrelin hormone (3.370kda) band was also visible between the bands of insulin B chain (3.5kda) and insulin A chain (2.5kda). Both hormones (IGF-1 and ghrelin) were detected in the blood of untreated buffaloes.



**Figure 2.** Gel of blood plasma from treatment 2 after electrophoresis.



**Figure 3.** Gel of blood plasma from treatment 1 after electrophoresis.

According to Kojima et al. [8] ghrelin is small synthetic molecules called growth-hormone secretagogues (GHSs) that stimulate the release of growth hormone (GH) from the pituitary. On the otherhand, Insulin-like Growth Factor – 1 (IGF-1) is the mediator of the anabolic and mitogenic activity of GH [13]. High protein content of Moringa will stimulate the acinar cells of the stomach to release ghrelin which is a hunger stimulating peptide hormone. Ghrelin will activate the hypothalamic pituitary gland to release growth hormone and insulin-like growth factor-1 that are involved in partitioning the energy and nutrients towards milk production.

#### 4. Conclusion

Feeding of concentrates with MoLM to dairy buffaloes had no effect in terms of milk yield and composition. Both hormones (putative IGF-1 and ghrelin) were detected in the blood of untreated buffaloes. Longer feeding trials must be done is to test the long term effects of moringa in milk yield and quality and strategy on coating of MoLM is suggested to protect and reduce the MoLM from fast degradation in the rumen, reticulum and omasum and to reach the abomasums where ghrelin is produced and to maximize its effect in milk production.

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