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# Effect of *Moringa oleifera* leaves powder in diets of lactating buffaloes

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## Abstract

**Background** Egyptian water buffalo as a dairy animal is adapted to the environmental conditions in Egypt and most of it is with small farmers, therefore, it was necessary to pay attention to improve its nutrition, increase its milk production, and improve its components by adding some feedstuffs of high nutritional value as *Moringa oleifera* which is a rapidly growing plant that is planted in tropical and subtropical area, in addition its leaves have high nutritive value, and it contains anti-oxidative and bioactive compounds, low anti-nutrient content and high amount of magnesium, which has positive effects on milk yield.

**Results** The results revealed that, daily milk or 4% FCM yields was significantly ($P < 0.05$) highest for diet supplemented by 50 g *Moringa olifera* leaves powder (MOLP, R2) compared with the other groups. By increasing MOLP supplementation level there was a significant ($P < 0.05$) decrease in daily milk yield than control. Milk contents of protein, fat and total solids were significantly ($P < 0.05$) increased, but lactose or solid not fat contents were significantly ($P < 0.05$) decreased than control with increasing MOLP level. The highest values of dietary nutrients digestibility and nutritive values (TDN and DCP%) were recorded with R2 and the lowest were recorded for R4. Blood serum total protein, albumin and globulin contents were significantly ($P < 0.05$) higher with feeding MOLP supplemented diets than control particularly for R2 and R3. While, there were significant ($P < 0.05$) decrease in urea, glucose and cholesterol but creatinine, AST and ALT were gradually increased with increasing MOLP level. Rations supplemented with MOLP was associated with obvious increase ($P < 0.05$) of antioxidant enzymes (GR, GPx, Cat and SOD) and decrease free radicals and the effect was more pronounced with increasing the supplementation level.

**Conclusions** These results indicated that 50 g *Moringa olifera* leaves powder supplementation to the diets of milking buffaloes improved milk yield, milk composition, nutrients digestibility, nutritive value and total antioxidant capacity.

**Keywords** *Moringa oleifera* leaves, Lactating buffaloes, Milk yield and constituents, Antioxidant enzymes

## Background

The total numbers of Egyptian buffaloes is about 3.95 million and most of it are owned by small holders and it represents 65–70% of total milk production, Egyptian buffaloes produce about 40.06% and 49.01% of the national meat and milk production, respectively (MALR 2017).

There are two types of Water buffaloes, swamp buffaloes and river buffaloes and it mostly adapted to highly fluctuations in weather temperature above 46 °C in the summer and fall below 4 °C at the winter this mean it considered adapted to the environmental conditions in developed country specially Egypt (Omran 2021). Borghese (2005) reported that milk yield of Egyptian buffaloes ranged between 1200 and 2100 kg/ head at 210 and 280 day for lactation duration with 6.5–7.0% milk

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fat. The peak period of milk production makes animals more prone to oxidative stress with enormous production of reactive oxygen species (ROS) that results in a disturbance of the balance between oxidant and antioxidant defense systems of the body (Obrador et al. 2019). All biomolecules including lipids, carbohydrates, and proteins are adversely affected by oxidative stress, which ultimately leads to a decline in productive performance (Vitale et al. 2018), therefore, it is necessary to use some feed additives to reduce oxidative stress, such as Moringa leaves which have antioxidant effect (Al-Juhaimi et al. 2020).

*Moringa oleifera* can be grown in tropical and subtropical regions in all types of soils that it is a drought tolerant plant and tolerate dry seasons up to 6 months (Su and Chen 2020). Moringa leaves are rich in minerals like calcium, iron, potassium and multivitamins, which are essential for animal performance and milk production and a good source of proteins, 22.99–29.36% (Sultana 2020), with about 47% bypass protein and with adequate amino acid profile, that can increase the rumen microbial protein synthesis (El-Naggar et al. 2017; Su and Chen 2020). *Moringa oleifera* leaves meal is rich in protein so it can be used as supplement to increase milk production (kholif et al. 2019).

There are a little informations about the impact of using MOLP in buffalo feeding specifically in milk production. In relation to this, the study was focused on investigating the effects of MOLP on milk yield and milk composition.

## Methods

### Site of work and *Moringa olifera* leaves preparation

This work implemented in the experimental animal farm of Animal Production Research Institute, Ministry of Agriculture which is located in Noubaria and the chemical analysis were in laboratories of the Animal Production Department of National Research Centre. Fresh *Moringa oleifera* leaves were brought from a private plant farm of newly reclaimed sandy soil and were left to dry in an open shaded area with daily shuffling upside down until completely dry then were grinded through 1 mm mesh screen.

### Experimental lactating buffalo's management and feeding program

The feeding experiment lasted 8 weeks, 16 healthy heads of Egyptian water buffaloes on their second and third lactation season were used to evaluate the effect of different supplementation levels of *Moringa oleifera* leaves powder (MOLP) on lactation performance, nutrients digestibility, blood biochemical constituents and enzymatic antioxidants activity. Two weeks before starting the experiment, buffaloes were injected against internal and external parasites and rottenly vaccinated against seasonally infectious diseases. All animals were housed in an open shaded yard surrounded by steel fences and supplied with internal steel barriers to separate between animal groups. Buffaloes in their mid-lactation season (150–160 days post-partum) weighed 600.0 ± 27.4 kg with an average daily milk production of 7.5–9.0 kg were blocked by previous milk records and body weight into four equal groups (4 each). Then, the experimental animal groups were randomly assigned to fed one of the experimental rations consisted of 7.0 kg concentrate feed mixture (CFM) + 15.0 kg corn silage + 4.0 kg rice straw (RS) per head. Whereas, R1 is ration based on uniform diet (0 MOLP) served as control group and R2, R3 and R4 were supplemented with by 50, 100 and 200 mg MOLP/head/day, respectively. Feed amounts were sufficient to provide energy and protein needed for lactating buffaloes (BÜL-BÜL 2010). Mechanically MOLP was mixed with CFM at the different tested levels and the amounts were prepared once for the whole feeding period. All diets offered daily in two equal portions at milking times (7.00 A.M. and 14.00 P.M.), corn silage and RS were offered once at 10.00 A.M., while drinking water in basins was available all the day round. Milk yield were daily recorded in the morning and evening milking and proportional composite samples of morning and evening milking were collected individually every week for chemical analysis. On the 8th week of the experiment, individual grab fecal samples were collected to determine nutrients digestibility by the Acid-insoluble ash technique. Whole blood samples were individually collected before feeding to determine blood serum biochemical composition, erythrocytic antioxidant enzymes activity and MDA content.

Chemical composition of experimental feed-stuffs (Table 1) and chemical composition of Moringa leaves (Table 2) was determined according to AOAC (2019), fiber fraction was analyzed according to Van Soest (1994). Macro minerals (Ca, P, K and Mg) and micro minerals (Mn,Zn,Fe and Cu) were determined according to Bouzid et al. (2015).

Essential amino acids were estimated by high performance liquid chromatography (HPLC) method (Dai et al. 2014).

Phytochemical compounds of dry Moringa leaves were estimated in ethanolic extract, where Moringa leaves were soaked overnight in 70% ethanolic solution at solid material to ethanol ratio of 1:3 (w/v). The suspension was filtered, then the filtrate was evaporated by a rotary evaporator and the extract was used to determine different measured compounds. Total chlorophyll and total carotenoids content were spectrophotometrically estimated according to AOAC (2019) and a standard β-carotene was used to create calibration curve. Total phenolic

**Table 1** Chemical composition of experimental feed-stuffs as dry matter basis (DM)

| Item | Moisture, % | DM composition, % | | | | | |
|---|---|---|---|---|---|---|---|
| | | OM | CP | CF | EE | NFE | Ash |
| CFM | 10.56 | 93.67 | 17.15 | 7.94 | 2.17 | 66.41 | 6.33 |
| Whole corn silage | 70.44 | 90.57 | 8.13 | 23.83 | 1.72 | 56.86 | 9.43 |
| Rice straw | 11.42 | 83.05 | 3.20 | 36.33 | 0.85 | 42.72 | 16.95 |

*CFM* concentrates feed mixture consisted of: 40% ground yellow corn, 6% wheat bran, 10% sugar beet pulp, 20% soya bean meal, 10% un-decorticated cotton seed meal, 5% sun flower meal, 5% cane molasses, 2% lime stone, 1.5% sodium chloride and 0.5 Vit. A + D3 + E (product of multi vita company of animal nutrition)

**Table 2** Chemical composition, amino acids profile and phytochemical compounds of *Moringa olifera* leaves

| Moisture | OM | CP | CF | EE | Ash | NFE | NDF | ADF | ADL |
|---|---|---|---|---|---|---|---|---|---|
| *Chemical composition, % (as DM basis)* | | | | | | | | | |
| 10.23 | 87.55 | 27.36 | 9.81 | 8.34 | 12.45 | 42.04 | 12.02 | 10.21 | 4.94 |
| **Ca** | **P** | **K** | **Mg** | **Mn** | **Zn** | **Fe** | **Cu** | | |
| *Measured macro-elements, %* | | | | Measured micro-elements, mg/Kg | | | | | |
| 3.71 | 0.37 | 1.38 | 0.48 | 48.60 | 40.01 | 558.7 | 6.35 | | |
| **Threonine** | **Methionine** | **Leucine** | **Isoleucine** | **Lysine** | **Histidine** | **Tryptophan** | **Phenyl-alanine** | **Valine** | |
| *Essential amino acids profile, g/100 g DM* | | | | | | | | | |
| 1.26 | 0.12 | 1.96 | 0.87 | 1.63 | 0.78 | 0.44 | 1.78 | 0.90 | |
| **Total chlorophyll, g/Kg DM** | **Total carotenoids, g/Kg DM** | **Total phenols, mg/g DM** | **Total flavonoids, mg/g DM** | | | | | | |
| *Some phytochemical compounds* | | | | | | | | | |
| 9.86 | 1.35 | 41.35 | 22.56 | | | | | | |

compounds and total flavonoids were estimated spectrophotometrically according to Ordonez et al. (2006).

Nutrient's digestion coefficients of experimental diets were determined by the acid insoluble ash technique outlined by McCarthy et al. (1977). Milk composition concerning fat content was determined according to Badertscher et al. (2007). Milk total solids (TS), total protein and ash were determined according to the standard methods of AOAC (2019). Lactose content was determined by the rapid test as described by John et al. (1957). Solids not fat (SNF) was estimated by difference. Fat corrected milk (4% FCM) was calculated according to Gaines (1923) equation:

$$4\%\text{FCM yield} = 0.4\text{M} + 15.0\text{F},$$

where M = milk yield and F = fat yield.

Blood samples (5 ml) were individually collected from the jugular vein in sterilized glass tubes, centrifuged at 3500 rpm for 15 min. to separate serum from the other blood contents then stored in clean sterilized tubes at −18 °C until further analysis. Collected blood erythrocytes were homogenized and kept under −40 °C to determine the enzymatic antioxidants activity. Serum total protein, albumin, urea, glucose, creatinine, cholesterol, alanine amino transferase (ALT) and aspartate aminotransferase (AST), serum malonaldehyde (MDE), Erythrocyte's antioxidants, Glutathione reductase (GR), Glutathione peroxidase (GPx), Catalase (CAT) and Superoxide dismutase (SOD) were determined calorimetrically (Biodiagnostic, Egypt), while globulin was calculated as the difference between total protein and albumin.

### Statistical analysis

Collected data of measured parameters were subjected to one-way analysis of variance applying the general linear model of SAS (2007).

## Results

Data in Table 3 represented milk chemical composition of buffaloes fed experimental diets and showed that there were significantly ($P<0.05$) increases in protein and total solids with Moringa leaves supplementation compared with control. While, there was a significant ($P<0.05$) decrease in milk lactose content for MOLP groups compared with control and a significant increase in fat content with feeding R3 and R4 compared with R1 while, feeding R2 didn't significantly

**Table 3** Milk chemical composition of experimental buffaloes (means ± SE)

| Item | Experimental groups | | | |
|---|---|---|---|---|
| | R1 | R2 | R3 | R4 |
| Protein | $3.21^{d} \pm 0.01$ | $3.46^{c} \pm 0.05$ | $3.61^{b} \pm 0.03$ | $3.78^{a} \pm 0.06$ |
| Fat | $6.48^{c} \pm 0.04$ | $6.71^{bc} \pm 0.08$ | $6.97^{b} \pm 0.09$ | $7.28^{a} \pm 0.11$ |
| Ash | 0.75 ± 0.02 | 0.77 ± 0.03 | 0.75 ± 0.02 | 0.74 ± 0.02 |
| Total solids | $13.93^{c} \pm 0.06$ | $14.13^{b} \pm 0.06$ | $14.39^{ab} \pm 0.08$ | $14.44^{a} \pm 0.08$ |
| Solids not fat | $7.45^{a} \pm 0.05$ | $7.42^{a} \pm 0.12$ | $7.42^{a} \pm 0.07$ | $7.16^{b} \pm 0.03$ |
| Lactose | $3.49^{a} \pm 0.04$ | $3.18^{b} \pm 0.13$ | $3.06^{c} \pm 0.08$ | $2.64^{d} \pm 0.08$ |

[a,b,c,d] Means within rows followed by different superscripts are significantly different at ($P < 0.05$)

affect milk fat content compared with R1. Insignificantly differences among R1, R2 and R3 feeding groups in solid not fat content were observe while, it was significantly decreased with R4. There was insignificant difference in ash milk content among groups. The highest milk protein and fat % were with buffaloes fed R4 compared with other groups, while there was no significant difference in milk fat % between buffaloes fed R2 and R3 and between R1 and R2.

Milk total solids % were insignificant difference between buffaloes fed R3 and R4 and between those fed R2 and R3, and the lowest value recorded with fed R1. Feeding buffaloes R4 significantly recorded the lowest milk solids not fat and lactose compared with other groups.

Data in Table 4 showed that R2 significantly ($P < 0.05$) increased milk yield and fat corrected milk (FCM 4%, kg) compared with other groups, while there was no significant difference in milk yield between R3 and R4 but significantly less than R1, and there was no significant difference in FCM between R1 and R3 but significantly less than R4.

Data of milk constituent's yield, g/h/d, showed that milk fat yield was significantly high with R2 followed by R4 and the lowest value ($P < 0.05$) observed in R1 and R3 without significant differences between them. R2 recorded the highest value ($P < 0.05$) of Milk protein yield followed by R3 and R4 and the lowest value ($P < 0.05$) was in R1. The highest milk lactose yield ($P < 0.05$) recorded in R1 and R2 followed by R3 and R4 respectively. Milk total solids yield of buffaloes fed R2 recorded the highest value ($P < 0.05$) compared with other groups and there were no significant difference among R1, R3 and R4.

Data in Fig. 1 showed that supplemented 50 g MOLP (R2) significantly increased daily milk yield, kg, continuously compared to other groups during the tested lactation period.

Data in Table 5 mentioned that feeding R2 significantly ($P < 0.05$) enhanced all nutrients digestibility as OM, CP, EE, CF and NFC and nutritive value as TDN and DCP compared to other groups, followed by R3 and the lowest value recorded with R1 and R4.

Data in Table 6 recorded that blood total protein (TP) g/dl, albumin g/dl and globulin g/dl were significantly ($P < 0.05$) high for buffaloes fed R2 and R3 compared with R1 and R4, and R4 was higher ($P < 0.05$) in TP and albumin compared to R1, and there was no significant difference in globulin among R2, R3 and R4 and between R1 and R4.

Control group recorded the highest value ($P < 0.05$) of blood urea mg/dl and cholesterol mg/dl compared with other groups followed by R2, R3 and R4 respectively. The lowest value ($P < 0.05$) of blood creatinine mg/dl found by R1 and R2 followed by R3 and the highest value recorded with R4. The highest value ($P < 0.05$) of blood glucose mg/dl recorded with R1 and R2 followed by R3 and the lowest value recorded by R4. The lowest blood AST and ALT IU/dl recorded with R2 and R3 followed by R1 and the highest value recorded with R4.

**Table 4** Milk and milk constituents yield of experimental buffaloes (means ± SE)

| Item | Experimental groups | | | |
|---|---|---|---|---|
| | R1 | R2 | R3 | R4 |
| Milk yield, Kg | $7.54^{b} \pm 0.12$ | $8.48^{a} \pm 0.41$ | $7.10^{c} \pm 0.31$ | $7.13^{c} \pm 0.39$ |
| FCM 4%, Kg | $10.35^{c} \pm 0.10$ | $11.94^{a} \pm 0.58$ | $10.26^{c} \pm 0.37$ | $10.65^{b} \pm 0.38$ |
| Yield of milk constituents, g/h/day | | | | |
| Fat | $488.8^{c} \pm 78.22$ | $569.7^{a} \pm 54.13$ | $494.9^{c} \pm 94.82$ | $519.8^{b} \pm 71.53$ |
| Protein | $241.9^{c} \pm 31.67$ | $293.7^{a} \pm 43.96$ | $256.7^{b} \pm 54.62$ | $269.3^{b} \pm 43.51$ |
| Lactose | $263.6^{a} \pm 27.61$ | $266.4^{a} \pm 48.57$ | $214.1^{b} \pm 39.86$ | $185.0^{c} \pm 38.76$ |
| Total solids | $1051.0^{b} \pm 93.14$ | $1195.5^{a} \pm 58.27$ | $1019.1^{b} \pm 62.13$ | $1026.9^{b} \pm 61.47$ |

[a,b,c] Means within rows followed by different superscripts are significantly different at ($P < 0.05$)

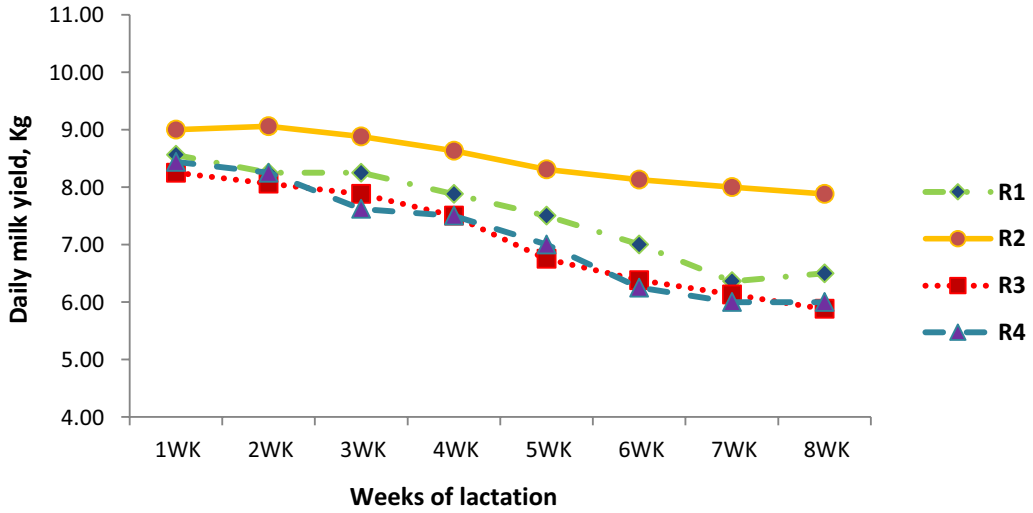


**Fig. 1** Lactation curve of different buffalo groups fed experimental diets

**Table 5** Nutrients digestibility (%) and nutritive value of experimental diets (means ± SE)

| Item | Experimental groups | | | |
|---|---|---|---|---|
| | R1 | R2 | R3 | R4 |
| OM | $63.51^{b} \pm 1.35$ | $68.34^{a} \pm 1.01$ | $64.26^{b} \pm 0.96$ | $58.36^{c} \pm 2.31$ |
| CP | $57.37^{c} \pm 0.72$ | $64.12^{a} \pm 0.61$ | $61.57^{b} \pm 0.84$ | $56.98^{c} \pm 1.67$ |
| EE | $68.65^{c} \pm 0.79$ | $72.96^{a} \pm 0.63$ | $71.16^{b} \pm 1.03$ | 70.64ab ± 0.82 |
| CF | $59.51^{c} \pm 1.12$ | $65.31^{a} \pm 1.31$ | $61.44^{b} \pm 2.28$ | $56.35^{d} \pm 0.91$ |
| NFE | $70.32^{b} \pm 0.86$ | $75.08^{a} \pm o.80$ | $74.15^{a} \pm 0.95$ | $68.12^{c} \pm 1.41$ |
| Nutritive value, % | | | | |
| TDN | $61.91^{c} \pm 0.56$ | $66.15^{a} \pm 0.24$ | $64.51^{b} \pm 0.87$ | $59.52^{d} \pm 1.18$ |
| DCP | $6.23^{c} \pm 0.15$ | $7.00^{a} \pm 0.06$ | $6.77^{b} \pm 0.11$ | $6.33^{c} \pm 0.21$ |

$^{a,b,c,d}$ Means within rows followed by different superscripts are significantly different at ($P < 0.05$)

Data of Table 7 represented that different levels of Moringa leaves supplementation decreased ($P < 0.05$) MDA (µmol MDA/L) value and increased ($P < 0.05$) glutathione reductase (GR) µmol/µg of erythrocyte protein, and catalase (CAT) µmol/µg of erythrocyte protein compared with control group (0 MOLP) and the highest GR value ($P < 0.05$) was recorded with R3 and R4 with no significant difference between them. R4 recorded the highest superoxide dismutase (SOD) U/µg of erythrocyte protein followed by R2 and R3 and the lowest value was with R1.

## Discussion

Data of Table 2 demonstrated that *Moringa olifera* leaves powder (MOLP) have high total phenolic compounds as total phenols and flavonoids which could improve buffaloes' health as well as preventing oxidation and enhancing shelf life of buffaloes' products such as milk, and have high antioxidant activity which can be used to scavenge free radicals of animals (Mbikay 2012).

The increases in milk protein and fat content with MOLP supplementation (Tables 3, 4) are in agreement with the findings of Kholif et al. (2018), hence MOLP stimulates the production of acetate which acts for the biosynthesis of fat (Babiker et al. 2016). However, the milk solids not fat content was not affected by 50 and 100 g MOLP supplementation and decreased with 200 g supplementation, these results consistent with which reported by Al-Juhaimi et al. (2020) for Aardi goats.

The high amount of protein in MOLP enhances the synthesis of selenocysteine based selenoproteins, these proteins have been reported to play a role in the modification of antioxidant defense system and improvement of milk production (Mehdi et al. 2013).

**Table 6** Blood serum biochemical constituents of buffaloes fed experimental diets (means ± SE)

| Item | Experimental groups | | | |
|---|---|---|---|---|
| | R1 | R2 | R3 | R4 |
| Total protein, g/dl | $6.63^{c} \pm 0.18$ | $7.59^{a} \pm 0.09$ | $7.62^{a} \pm 0.10$ | $7.11^{b} \pm 0.97$ |
| Albumin, g/dl | $3.12^{c} \pm 0.03$ | $3.48^{a} \pm 0.04$ | $3.50^{a} \pm 0.06$ | $3.26^{b} \pm 0.03$ |
| Globulin, g/dl | $3.51^{b} \pm 0.05$ | $4.11^{a} \pm 0.20$ | $4.12^{a} \pm 0.04$ | $3.85^{ab} \pm 0.07$ |
| Urea, mg/dl | $30.51^{a} \pm 1.61$ | $26.42^{b} \pm 0.37$ | $25.16^{b} \pm 1.11$ | $22.64^{c} \pm 0.72$ |
| Creatinine, mg/dl | $1.21^{c} \pm 0.02$ | $1.17^{c} \pm 0.03$ | $1.45^{b} \pm 0.08$ | $1.68^{a} \pm 0.13$ |
| Glucose, mg/dl | $64.85^{a} \pm 1.19$ | $62.95^{a} \pm 1.36$ | $56.37^{b} \pm 1.28$ | $52.08^{c} \pm 0.79$ |
| Cholesterol, mg/dl | $95.03^{a} \pm 1.89$ | $83.67^{b} \pm 2.78$ | $82.29^{b} \pm 1.64$ | $81.33^{b} \pm 1.16$ |
| AST, IU/dl | $42.83^{c} \pm 2.10$ | $43.52^{b} \pm 2.73$ | $45.84^{b} \pm 1.89$ | $51.30^{a} \pm 1.06$ |
| ALT, IU/dl | $15.56^{c} \pm 0.65$ | $15.82^{b} \pm 1.13$ | $16.04^{b} \pm 0.89$ | $18.43^{a} \pm 1.20$ |

[a,b,c] Means within rows followed by different superscripts are significantly different at ($P < 0.05$)

The improvement in nutrients digestibility and nutritive value (Table 5) with low level of MOLP supplementation was consistent with the results of previous studies (Kholif et al. 2019; Parra-Garcia et al. 2019; Dhanasekaran et al. 2020; Abdel-Raheem and Hassan 2021). There are several possible explanations with supplemented 50 g MOLP improvement of nutrient digestibility, that rumen microflora can utilize low levels of secondary metabolites (e.g., phenolics, tannins, saponins and essential oils) from Moringa oleifera leaves and use them as energy sources without any negative effects on rumen fermentation while, the higher levels of Moringa leaves seem to negatively affect rumen microorganisms due to their antimicrobial properties (Bodas et al. 2012).

Usually, blood parameters are used to assess the overall health and vitality of animals. In this study dietary supplementation of MOLP to buffaloes significantly influenced all studied blood constituents (Table 6) but all of them were within the normal reference ranges (Boyd 2011).

Effect of MOLP supplementation on total protein, albumin, globulin, creatinine, blood urea nitrogen, glucose and total cholesterol are consistent with found by Abdel-Raheem and Hassan (2021).

It is evident from the findings of this study that MOLP supplemented groups had high level of serum total protein (TP) as compared to the control group, the high value of TP may be to satisfy the high protein requirements of milk production, and these results supported by the findings of other studies in sows and Jersey cattle (Kekana et al. 2019; Sun et al. 2020).

The decrease in serum glucose level for MOLP rations might be due to that *Moringa oleifera* is known as one of the highly potential anti diabetic plants possibly potentiating the insulin action (Farooq et al. 2007). However, serum glucose level was in normal range (50–75 mg/100 mL) required for a healthy buffalo (Pal et al. 2010).

The herein presented results of decreasing serum cholesterol of buffaloes fed diets supplemented with MOLP are agreed with reported by Kholif et al. (2016) in lactating goats, Zeng et al. (2018) in lactating dairy cows and Al-Juhaimi et al (2020) in Aardi goats. The significant decrease in serum cholesterol level in samples from buffaloes fed MOLP may have resulted from functional effects associated with phenolic and saponin contents. Saponins and antioxidants can reduce cholesterol synthesis and its absorption (Saxena et al. 2013).

The serum of buffaloes fed diets supplemented with MOLP shown in Table 7 the Moringa supplementation significantly enhanced the serum total antioxidant capacity (TAC) by declined the non-enzymatic antioxidant activity as malondialdhyde concentration (MDA) and increased enzymatic antioxidant activity as glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities

**Table 7** Enzymatic and non-enzymatic antioxidants activity of lactating buffaloes in experimental groups (means ± SE)

| Item | Experimental groups | | | |
|---|---|---|---|---|
| | R1 | R2 | R3 | R4 |
| MDA (μmol MDA/L) | $5.46^{a} \pm 0.88$ | $2.68^{b} \pm 0.07$ | $2.32^{b} \pm 0.08$ | $2.29^{b} \pm 0.14$ |
| GR (glutathione reductase), μmol/μg of erythrocyte protein | $0.260^{c} \pm 0.012$ | $0.385^{b} \pm 0.005$ | $0.424^{a} \pm 0.007$ | $0.436^{a} \pm 0.010$ |
| GPx (glutathione peroxidase), μmol/μg of erythrocyte protein | $1.184^{d} \pm 0.020$ | $1.705^{c} \pm 0.030$ | $1.800^{b} \pm 0.018$ | $1.894^{a} \pm 0.033$ |
| CAT (catalase), μmol/μg of erythrocyte protein | $0.26^{b} \pm 0.01$ | $0.41^{a} \pm 0.01$ | $0.43^{a} \pm 0.01$ | $0.44^{a} \pm 0.01$ |
| SOD (superoxide dismutase), U/μg of erythrocyte protein | $4.50^{c} \pm 0.19$ | $7.51^{b} \pm 0.16$ | $7.77^{b} \pm 0.19$ | $8.17^{a} \pm 0.17$ |

[a,b,c,d] Means within rows followed by different superscripts are significantly different at ($P < 0.05$)

compared to the control group. The decreasing of MDA values may be due to the high content of phenolic compounds in Moringa leaves (Al-Juhaimi et al. 2020) and its antioxidant activity (Babiker et al. 2018). So, both enzymatic and non-enzymatic antioxidant components defense system work in collaboration to maintain the lactating animals in suitable condition by reducing the stress resulting from lactation (Gong and Xiao 2018). Also, Babiker et al. (2016) for Najdi ewes reported that the high content of catalase may contributes to the reduction of MDA value by the decomposition of hydroperoxides, thereby protecting the milk by oxidation from further spoilage, this could help in enhancing the shelf life of buffaloes' milk and its products.

## Conclusions

These results indicated that 50 g *Moringa olifera* leaves powder supplementation to the diets of milking buffaloes improved milk yield, milk composition, nutrients digestibility, nutritive value and total antioxidant capacity. Also, we can be concluded that MOLP could improve the immune system of animals through transfer of bioactive compounds specially antioxidants from fodder to milk, and the high level of Moringa leaves not recommended because it seems to be negatively affect rumen microorganisms due to their antimicrobial properties which affect the utilization of nutrients and reflected on the decrease in milk production.

### Abbreviations

| | |
|---|---|
| MOLP | Moringa *oleifera* leaves powder |
| CFM | Concentrate feed mixture |
| DM | Dry matter |
| CP | Crude protein |
| CF | Crude fiber |
| EE | Ether extract |
| OM | Organic matter |
| NFE | Nitrogen-free extract |
| TDN | Total digestible nutrients |
| DCP | Digestible crude protein |
| N | Nitrogen |
| NDF | Nutrient detergent fiber |
| ADF | Acid detergent fiber |
| ADL | Acid detergent lignin |
| AST | Aspartate aminotransferase |
| ALT | Alanine aminotransferase |
| MDA | Serum malonaldehyde |
| GR | Glutathione reductase |
| GPx | Glutathione peroxidase |
| CAT | Catalase |
| SOD | Superoxide dismutase |

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### Author contributions

AYE and MHMY had proposed the scientific idea and the experimental design. AAH and MSK were responsible about field trials. SE was participated in collecting and analyzing diets, feces, urine and blood samples. AYE, MHMY and SE contributed in tabulating the results according to the mathematical model applied and writing final manuscript. All authors have read and approved the manuscript.

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### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

## Declarations

### Ethics approval and consent to participate

This study was conducted in strict accordance with the provisions of the relevant Egyptian laws and with Helsinki Declaration, good medical and laboratory practice (GCP and GLP) guidelines as well as Institutional Animal Care and Use committee (IACUC) guidelines and recommendations and World Health Organization (WHO) rules regarding ethics of scientific research. The protocol was approved by the Medical Research Ethics Committee of the National Research Centre and the final approval hold the number (2482032022).

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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