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Short communication

# Daily and alternate day supplementation of *Moringa oleifera* leaf meal or soyabean meal to lambs receiving oat hay

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ABSTRACT

We hypothesized that *Moringa oleifera* foliage which is high in crude protein and almost free in main secondary compounds could replace soyabean meal used in supplementation strategies of livestock. Therefore, this experiment aimed to evaluate the effect of daily and alternate day supplementation of Moringa leaf meal (MLM) or soybean meal (SBM) to sheep on intake and digestion and glucose, protein and urea in blood plasma. Twenty Barbarine male lambs (average initial body weight, $27.3 \pm 3.0$ kg), were randomly divided into four groups of five lambs each. They were adapted for 22 days to dietary treatments before starting a 6-day total collection period. All animals consumed oat hay *ad libitum* supplemented with concentrates composed of barley grain only (C-BAR) or mixed with SBM (C-SBM), or MLM (C-MLM) as protein sources. Groups 1 and 2 received daily C-SBM and C-MLM, respectively. However, groups 3 and 4 had access to concentrates containing the protein sources (C-SBM and C-MLM) with alternate day that means they received for one day these concentrates and in the following day they received barley grain only (C-BAR). Soybean meal and MLM incorporation in concentrates had similar effects ($P > 0.05$) on water, hay, and digestible organic matter (OM) intakes and DM, organic matter (OM) and neutral detergent fiber (NDF) digestibility. Daily substitution of SBM by MLM increased ($P = 0.024$) crude protein (CP) intake and apparent total tract digestibility. However, the alternate day supplementation decreased apparent total tract CP digestibility but increased CP intake for SBM treatment. The concentration of ruminal ammonia nitrogen before feeding was higher ($P = 0.012$) in groups 1 and 3 than in the other groups. The concentration of blood glucose was similar ($P > 0.05$) among treatments. However, lambs receiving C-SBM exhibited highest concentrations of protein ($P = 0.039$) and urea ($P = 0.001$) in the blood. It is concluded that MLM administered at two day-intervals had similar effects on feed intake, digestion and blood metabolites to SBM incorporated in concentrate distributed to sheep receiving oat hay.



## 1. Introduction

Livestock reared in dry areas is facing feed shortage and fed on low quality diets. These constraints are impacting negatively on their performances. Therefore, the development of cost-effective supplementation strategies is targeted in

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these areas. Barley cropping is a common practice in North Africa and is contributing to a large extent in energy supplementation of ruminants. However, the main protein source used in livestock feeding in Tunisia and in many other countries is soyabean meal (SBM) which is expensive, thus not easily affordable to small-scale farmers. Soybean meal is widely used in livestock feeding as it improved productive and reproductive performances of ruminants (Molle et al., 1997). Cozzi et al. (1995) showed that feather and blood meal can partially replace SBM in diets of sheep. However, the FAO has recommended banning the use of mammalian meat and bone meal as protein sources in ruminant feeding as this may have a negative impact on human health (Food and Agriculture Organization, 2001). Therefore, the use of local legume plants as alternative protein supplements is recommended. Malunga et al. (2009) concluded that lambs receiving SBM performed better than those receiving *Mucuna pruriens*. However, the cost to benefit ratio would favor the use of *Mucuna* instead of SBM. *Moringa oleifera* holds promise as an alternative protein supplement for ruminants. It yields high consumable biomass (24 t/ha/year) that is high in CP (193–264 g/kg DM) and true protein (Makkar and Becker, 1997). Studies on sheep response to *Moringa* foliage are scarce and the few literature data suggest that sheep (Murro et al., 2003) and goat (Manh et al., 2005) could benefit from this plant species. *Moringa oleifera* has been introduced in Tunisia in 2009. But, there is no information on the response of local sheep breeds to this plant species. Alternate supplementation of protein sources, *e.g.* soya bean meal, could be a solution to alleviate feeding cost (McGuire et al., 2013a,b). Therefore, we designed this experiment to determine the effect of daily and alternate day supplementation of SBM and MLM on feed and water intakes, diet apparent digestibility, ruminal fermentation and blood parameters in lambs. The comparison of sheep response to daily and alternate distribution of SBM or MLM-containing concentrates would indicate whether the use of these two protein sources could be reduced or not, thus whether the feeding cost could be alleviated or not.

## 2. Materials and methods

### 2.1. Study site and feeds

A digestibility trial was carried out at the National Institute of Agricultural Research of Tunisia (INRAT) in January–February 2011. Oat hay bales (18–20 kg) and barley grain were produced in the Experimental Station of INRAT at Bourbia. However, SBM and mineral and vitamin supplements were purchased from the local market. Air-dried Moringa leaves powder was prepared in the laboratory of the Animal and Forage Production of INRAT. Moringa plantation was established in the National Institute of Agricultural Research of Tunisia (INRAT) and harvested every 6 weeks. Then, *Moringa* leaves were air-dried for 2 weeks and crushed before mixing it with barley grain and the mineral and vitamin supplement. Three types of concentrates were used in this trial. All of them contained barley grain without or with soyabean meal (common protein source) or dried Moringa leaf meal (alternative protein source). The composition of these concentrates is reported in Table1.

**Table 1**
Proportions of ingredients in concentrates and chemical composition of feeds.[a]

| | C-SBM | C-MLM | C-BAR | Oat hay |
|---|---|---|---|---|
| Ingredients (g/kg) | | | | |
| Soybean meal | 190 | 0 | 0 | |
| Barley grains | 780 | 505 | 970 | |
| Dried Moringa leaves | 0 | 465 | 0 | |
| Mineral and vitamin supplement | 30 | 30 | 30 | |
| Chemical composition | | | | |
| DM (g/kg) | 880 | 880 | 880 | 950 |
| OM (g/kg DM) | 980 | 920 | 980 | 930 |
| CP (g/kg DM) | 135 | 158 | 113 | 26 |
| NDF (g/kg DM) | 250 | 270 | 273 | 770 |
| ME (MJ/kg DM) | 13.8 | 13.6 | 13.7 | 11.3 |
| Total phenols (g/kg DM)[b] | 3 | 11 | 2 | 6 |
| Total tannins (g/kg DM)[b] | 2 | 7 | 2 | 3 |
| Saponins (g/kg DM)[c] | 15 | 42 | 26 | 28 |

[a] C-SBM: soyabean meal-containing concentrate, C-MLM: Moringa leaf meal-containing concentrate, and C-BAR: barley concentrate.
[b] Equivalent tannic acid.
[c] Equivalent diosgenin.

### 2.2. Animals and treatments

Twenty adult Barbarine lambs (average initial body weight = 27.3 ± 3.0 kg) which had been treated with anthelmintics were selected on the basis of live weight and used in this study. The lambs were allocated to four groups of five animals each; group 1 and 2 received concentrates containing SBM (C-SBM) and MLM (C-MLM), respectively at daily frequency. However, groups 3 and 4 were supplemented by these concentrates (day $n$) and barley concentrate (C-BAR, day $n+1$) at alternate days for the whole period of this experiment. The feed ingredient, formulation and chemical composition are shown in Table 1. After a 21-days adaptation to dietary treatments (Day1–21), lambs were moved into individual metabolism crates for a 10 days digestibility trial (4 days for adaption to the new housing conditions (Day 22–Day 25) and 6 days for collection of excreta (Day 27–Day 31).

### 2.3. Sampling and measurements

Samples of SBM, MLM, barley grain and oat hay were collected daily throughout this experiment. Each sample was divided into two parts. One part was used for DM determination (80 °C) and the other was dried at 50 °C and used for chemical analysis. Blood samples were taken in the two first and the two last days of the digestibility trial (Days 26, 27, 30 and 31) from the jugular vein of each lamb before the distribution of the morning meal and centrifuged at 3000 × *g*. Plasma was recuperated and conserved at −20 °C until analyzed. Blood samples were analyzed for glucose, total proteins and urea using standard kits (Biomaghreb).

### 2.4. *in vivo* digestibility study

Lambs housed in metabolic cages were acclimated to the new housing conditions for four days before starting fecal collection over six consecutive days. Every day, the animals were given oat hay and clean water *ad libitum*.

Individual feed refusal and feces were weighed daily and a sample of 10% of the total weight was then collected. The samples of refusals or feces were pooled for each animal, were dried at 50 °C and then ground to pass through 1 mm screen prior to chemical analyses. Other samples of feed distributed and individual refusals and feces were dried at 80 °C until constant weight is achieved for DM content determination. Feces were daily collected at 09:00 h from each animal. Rumen fluid was withdrawn from each animal using stomach tube in the last day of total collection period immediately before the distribution of morning meal (0 h) then at 3 h post-feeding. After pH measurement on a digital pH-meter, the rumen fluid was filtered through 4 layers of cheesecloth, 1 ml samples were placed in Eppendorf tube then 3 drops of sulfuric acid (97%) were added in each tube before storing at −20 °C. Samples of rumen fluid were centrifuged (3000 × *g*) then analyzed for ammonia nitrogen (Weatherburn, 1967).

### 2.5. Gas production measurement

Rumen fluid was collected from two Barbarine rams (average live weight 45 ± 1.5 kg) fitted with rumen cannula before the morning meal using a manual pump, and transferred to the laboratory into pre-warmed thermos flasks. On arrival to the laboratory, it was filtered through two layers of cheese cloth and pooled. Rams were housed indoors and consumed a diet composed of oat hay *ad libitum* and 300 g of concentrate (barley 0.8 kg/kg and soybean meal 0.2 kg/kg) to fulfill maintenance requirements. Feed samples were weighted in triplicate (200 mg) into glass syringes before buffered rumen fluid was added (30 ml).

### 2.6. Laboratory analyses and calculations

Chemical analyses were carried out on the ground samples of feeds distributed, refusals and feces. These samples were analyzed for DM, organic matter, N, ash, acid detergent fiber (ADF) (AOAC, 1990), and neutral detergent fiber (NDF) according to Van Soest et al. (1991). Crude protein (CP) content was calculated as $N \times 6.25$. Ground samples (200 mg) of diet ingredients were extracted in duplicate with 10 ml 80 g/kg aqueous acetone or 10 ml 80 g/kg aqueous methanol solutions for a night at 4 °C. After centrifugation (3000 × *g* for 10 min at 4 °C), supernatants were used to analyze saponins (*g* equivalent diosgenin/kg DM) according to Hiai et al. (1976), total phenols and tannins (*g* equivalent tannic acid/kg DM) and condensed tannins (*g* equivalent leucocyanidin/kg DM) according to Makkar (2003). Metabolizable energy (ME) content of feeds was estimated using the equation of Menke and Steingass (1988):

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136Gp + 0.0057CP + 0.0002859CP^2$$

where CP is the crude protein in g/kg DM and Gp is the net gas production (ml) from 200 mg of substrate after 24 h of incubation.

### 2.7. Statistical analyses

Data on feed intake, diet digestibility, rumen pH and ammonia N concentration and blood metabolites were subjected to analyses of variance using the general linear model of SAS (1987). Results are reported as LSMEANS, significance was set at $P \leq 0.05$.

## 3. Results

### 3.1. Feed and water intakes and in vivo diet digestibility

Table 2 reports data on feed and water intakes and apparent digestibility of diets. Lambs from the three groups exhibited the same feed and water intakes. Neither the supplement type nor the alternate supplementation affected nutrients intakes. OM and NDF digestibility of diets were not affected by dietary treatments ($P > 0.05$).

**Table 2**
Feed and water intakes and in vivo apparent diet digestibility.

| | Oat hay + supplement | | | | S.E.M. | *P*-value Frequency | Source | Frequency × source |
|---|---|---|---|---|---|---|---|---|
| | SBM daily | MLM daily | SBM alternate | MLM alternate | | | | |
| Drinking water consumption | | | | | | | | |
| Liter/day | 1.7 | 1.4 | 1.5 | 1.5 | 0.11 | 0.490 | 0.707 | 0.225 |
| Liter/kg $BW^{0.75}$ | 0.13 | 0.12 | 0.12 | 0.12 | 0.010 | 0.537 | 0.506 | 0.204 |
| Hay intake (g/kg $BW^{0.75}$) | 47.1 | 49.6 | 51.3 | 49.0 | 3.46 | 0.982 | 0.618 | 0.502 |
| Diet digestibility (%) | | | | | | | | |
| DM | 61.5 | 61.1 | 60.0 | 62.3 | 1.41 | 0.510 | 0.894 | 0.346 |
| OM | 66.1 | 64.8 | 64.4 | 66.2 | 1.28 | 0.841 | 0.914 | 0.241 |
| CP | 41.7[a] | 44.8[a] | 34.2[b] | 46.7[a] | 2.71 | 0.010 | 0.312 | 0.101 |
| NDF | 53.7 | 58.5 | 54.3 | 56.6 | 2.38 | 0.151 | 0.791 | 0.609 |
| Nutrient intakes (g/kg $BW^{0.75}$) | | | | | | | | |
| DM | 72.8 | 74.9 | 77.8 | 75.7 | 1.40 | 0.435 | 1.000 | 0.570 |
| OM | 48.8 | 50.8 | 52.6 | 50.5 | 1.30 | 0.590 | 0.990 | 0.545 |
| CP | 4.7 | 5.3 | 4.7 | 5.1 | 0.11 | 0.595 | 0.102 | 0.445 |
| NDF | 41.7 | 44.7 | 45.0 | 44.2 | 1.80 | 0.625 | 0.708 | 0.506 |

BW, body weight.
[a] Means in the same raw followed by different letters are significantly different ($P < 0.05$).
[b] Means in the same raw followed by different letters are significantly different ($P < 0.05$).

**Table 3**
Ruminal pH and ammonia nitrogen concentration and blood metabolites.

| | Oat hay + supplement | | | | S.E.M. | *P*-value frequency | Source | Frequency × source |
|---|---|---|---|---|---|---|---|---|
| | SBM daily | MLM daily | SBM alternate | MLM alternate | | | | |
| Ammonia nitrogen (mg/dl) | | | | | | | | |
| 0 h–Day 1 | 10.9 | 9.6 | 10.6 | 6.8 | 1.2 | 0.261 | 0.035 | 0.248 |
| 3 h–Day 1 | 11.4[a] | 9.8[a] | 9.2[a] | 7.0[b] | 0.91 | 0.015 | 0.051 | 0.753 |
| 0 h–Day 2 | 11.7[a] | 11.0[a] | 8.7[a] | 5.1[b] | 1.13 | 0.001 | 0.078 | 0.218 |
| 3 h–Day 2 | 12.4 | 10.4 | 10.5 | 10.6 | 0.80 | 0.316 | 0.235 | 0.209 |
| pH | | | | | | | | |
| 0 h–Day 1 | 6.71 | 6.77 | 6.60 | 6.56 | 0.079 | 0.056 | 0.911 | 0.529 |
| 3 h–Day 1 | 6.18 | 6.36 | 6.16 | 6.18 | 0.056 | 0.091 | 0.091 | 0.169 |
| 0 h–Day 2 | 6.73[a] | 6.77[a] | 6.64[a] | 6.32[b] | 0.085 | 0.006 | 0.126 | 0.056 |
| 3 h–Day 2 | 6.18 | 6.32 | 6.26 | 6.04 | 0.062 | 0.146 | 0.525 | 0.011 |
| Blood parameters | | | | | | | | |
| Glucose (g/l) | 0.60 | 0.55 | 0.62 | 0.59 | 0.030 | 0.218 | 0.280 | 0.714 |
| Total proteins (g/l) | 68.9[ac] | 60.3[ac] | 68.5[a] | 72.8[bc] | 2.783 | 0.446 | 0.046 | 0.034 |
| Urea (g/l) | 0.30[a] | 0.22[b] | 0.21[b] | 0.16[b] | 0.017 | 0.001 | 0.001 | 0.549 |

[a] Means in the same raw followed by different letters are significantly different ($P < 0.05$).
[b] Means in the same raw followed by different letters are significantly different ($P < 0.05$).
[c] Means in the same raw followed by different letters are significantly different ($P < 0.05$).

The alternate supplementation of SBM resulted in a decrease ($P < 0.05$) of the diet CP digestibility.

### 3.2. Rumen fermentation

Rumen pH was not affected with treatments, *i.e.* protein source and distribution frequency (Table 3). Alternate supplementation of Moringa resulted in a decrease ($P < 0.05$) of ammonia–nitrogen concentration in the rumen before the distribution of the morning meal.

### 3.3. Blood parameters

Blood profile is reported in Table 3. Dietary treatments had no effect on glucose concentration ($P > 0.05$). Total proteins and urea concentrations were lower in lambs receiving Moringa daily than in those supplemented with SBM. However, alternate day supplementation of Moringa resulted in similar concentration of blood total proteins than the SBM treatment.

## 4. Discussion

There is a growing interest on *M. oleifera* plantation in many regions. The main benefits from this lesser-known tree species include the high yield and nutritional quality of consumable biomass considered as good source of true protein for livestock (Debela and Tolera, 2013; Makkar and Becker, 1996). The CP content of MLM used in the current work was 250 g/kg DM which is similar to that reported by Makkar and Becker (1996). Melesse et al. (2012) reported higher contents of CP (287 and 290 g/kg DM) and ME (9.63 and 9.56 MJ/kg DM) in Moringa leaves growing in mid and low altitudes (1700 and 1100 m, respectively above sea level). The quality protein in MLM noted by Makkar and Becker (1996) and Melesse et al. (2012) could justify the potential of MLM to replace oleaginous meals. Indeed, Moyo et al. (2012) evaluated the replacement value of Moringa leaves for sunflower cake and concluded that goats consuming these two nitrogen sources performed similarly. Alternate day supplementation of protein sources could be also another option to economize on their use without affecting livestock performances. To our knowledge, this is the first study on the response of sheep to alternate-day supplementation with C-MLM or C-SBM. Our experiment showed that there was no effect of alternate day supplementation on hay, digestible organic matter and water intakes and DM and OM digestibility of the diet. These findings are in line with those by McGuire et al. (2013a,b) who concluded that nitrogen source (SBM and urea) and supplementation frequency had little effect on intake and digestibility of low-quality hard fescue straw by lambs. However, there were significant decrease of diet CP digestibility for sheep receiving C-SBM at alternate day, while daily and infrequent access to C-MLM had no effect on this dependent variable. This could be the result of higher proportion of true protein and or saponins in Moringa foliage than SBM (Makkar and Becker, 1997).

Replacement of SBM with MLM in the concentrate had no effect on blood glucose. However, the concentrations of total protein and urea were higher in the plasma of lamb receiving C-SBM than lambs supplemented by C-MLM. Lamb receiving C-SBM had higher ruminal ammonia N than MLM-lamb; this difference is exacerbated in lamb subjected to alternate distribution of C-MLM. This phenomenon could be ascribed to differences in N solubility between SBM and MLM. According to literature, SBM is higher in rumen degradable crude protein (74.9%, Cozzi et al., 1995) than MLM (48.6%, Makkar and Becker, 1996). Atkinson et al. (2010) evaluated the effect of ruminal protein degradability and supplementation frequency on intake, apparent digestibility, N retention, and nutrient flux across visceral tissues of lambs fed a low-quality forage diet. They concluded that the release of ammonia N by the portal-drained viscera (PDV) was reduced in alternate-day-supplemented lambs compared with lambs

receiving rumen degradable protein daily. In our experiment, all treatments exhibited concentrations of ammonia N higher than the optimum level recommended by Satter and Slyter (1974) for normal microbial activity in the rumen. This reinforces our conclusion that MLM distributed at alternate day could be considered as a cost-effective option to replace SBM as concentrate feed for sheep.

## 5. Conclusion

This study showed that daily and alternate distribution of C-MLM had similar effect on feed intake, digestion and blood parameters in sheep than C-SBM. Therefore, when incorporated in concentrate in CP equivalent, MLM could, à priori, replace SBM. However, further work is required on growing lambs to confirm the replacement value of MLM for SBM and to determine the cost/benefit ratio of daily and alternate supplementation with these two protein sources.

## Conflict of interest statement

Authors of this manuscript certify that there is no conflict of interest.

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