Inclusion of Moringa oleifera leaf meal in the diet of locally bred chickens: effects on growth performance, semen and hatchability traits

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ABSTRACT
The presence of essential amino acids and low anti-nutritional factors (ANFs) in Moringa oleifera leaf meal (MOLM) designates it as a good source of nutrients for poultry. This study investigated the effect of dietary MOLM on the growth performance, semen, hatchability traits and day-old chick weight of Potchefstroom Koekoek (PK) chickens. Two isonitrogenous and isoenergetic diets were formulated by diluting basal layer and grower mash with 0 (MOLM0) and 70% (MOLM70) with MOLM. Eighty PK chickens (40 hens and 40 roosters) aged 22 weeks were used in this study. For growth performance and hatchability traits, 40 laying hens were randomly assigned to two treatments with four replicates having five laying hens per pen. Diets did not significantly affect growth performance. The MOLM70 diet resulted in increased sperm velocity (rapid), semen concentration, semen pH, motility and progressive motility than the control (MOLM0). It is concluded that the inclusion of MOLM in chicken diets did not adversely affect growth performance, but improved sperm motility, hatchability and weight of day-old chicks of PK chickens. The findings recommend that MOLM is nutritionally equipped to enhance both the reproductive and the productive performance of PK.

Introduction

In rural communities of Africa, locally bred chickens are sources of essential amino acids, fatty acids and micronutrients for the general populace (Manyeula et al. 2019). Yet, there has been a drop in the number of locally bred chickens, mainly due to their poor productive and reproductive performance (Ben Larbi et al. 2013). It has been known that low fertility and poor hatchability are experienced under natural mating conditions due to poor semen quality as results of poor nutrition (Bucak et al. 2010). Peters et al. (2008) reported that hydroethanolic extract from M. oleifera leaves significantly increased the number of interstitial Leydig cells in rats. Also, Abu et al. (2013) reported that MOLM had no adverse effect on the testicular morphometry and epididymal sperm quality of rabbit bucks at an inclusion level of up to 15%. Additionally, Syarifuddin et al. (2017) stated that MOLM increased plasma testosterone concentrations, libido and sperm motility of Bali bulls. Therefore, this study was aimed at evaluating the effect of MOLM on the growth performance, semen quality and hatchability traits of Potchefstroom Koekoek (PK) chickens. It was hypothesized that including MOLM in locally bred chicken diets would have no negative effects on growth performance, semen and hatchability traits.

Recently, leaf meals have been used in animal nutrition for their nutritional and medicinal benefits. Moringa oleifera is one of the plants that contain all of these compounds. Moringa oleifera leaf meal (MOLM) is best known for its high leaf protein (27%) content, adequate amino acid profile, high levels of vitamins A and E, low levels of anti-nutritional compounds, fatty acids (Ogbe and Affiku 2012; Sebola et al. 2015), phenol (8 μg/ml), flavonoids (27 μg/ml) (Rajanandh and Kavitha 2010), an alkaloid (0.07%) (Madukwe et al. 2013), ferulic acid (46.8 mg/g) and chlorogenic acid (18.0 mg/g) (Fitri et al. 2015). However, the use of MOLM as a reproductive booster in poultry is still limited. Prabatnightsoro et al. (2015) reported that hydroethanolic extract from M. oleifera significantly increased the number of interstitial Leydig cells in rats. Also, Abu et al. (2013) reported that MOLM had no adverse effect on the testicular morphometry and epididymal sperm quality of rabbit bucks at an inclusion level of up to 15%.

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Materials and methods

The study was conducted according to the guidelines of the Ethics. All animal husbandry practices were performed with full consideration of animal welfare.

Study area

During the experimental period, ambient temperatures ranged from 19°C to 25°C.

Feed ingredients and experimental diets

*Moringa oleifera* leaves were collected from Limpopo, to formulate two isonitrogenous and isoenergetic diets (Table 1) in a mash form to meet the nutritional requirements of growing chickens. The diets were as follows: MOLM0 = a basal diet without MOLM and MOLM70 = a basal diet with 70 g/kg MOLM (Table 1). Sebola et al. (2015) optimized a 70% MOLM inclusion rate which could be used without compromising the objective of meeting the nutritional requirements of growing locally bred chickens. The experiment was conducted in three parts.

Experiment 1A – growth performance

A total of 40 laying hens were raised on a starter mash for four weeks. At five weeks of age, the hens were randomly allotted to two experimental diets until 40 weeks of age. A completely randomized design (CRD) was used for this experiment with a pen holding five birds, replicated four times, resulting in a total of eight battery cages (measuring 60 × 50 × 75 cm). Feed and water were provided *ad libitum* during the experimental period under continuous lighting throughout the trial. Feed offered to the hens and refusals were weighed daily for nine weeks. Average weekly feed intake (AWFI), average weekly body weight gain (AWG) and feed conversion ratio (FCR) were calculated (Ogbe and Affiku 2012) and data were analysed following Model 2.

Table 1. Ingredients and nutrient composition (g/kg) of *Moringa oleifera* leaf meal (MOLM) based diets.

| Ingredients               | MOLM0 | MOLM70 |
|---------------------------|-------|--------|
| MOLM                      | 0     | 70.0   |
| Yellow maize              | 670.6 | 647.3  |
| Prime gluten 60           | 50.0  | 50.0   |
| Full fat soya meal        | 70.0  | 70.0   |
| Soya bean meal            | 85.3  | 58.2   |
| Sunflower oilcake         | 80.0  | 80.0   |
| Limestone powder          | 12.3  | 7.1    |
| Potassium carbonate       | 1.2   | 0.9    |
| Monocalcium phosphate     | 9.8   | 10.0   |
| Salt                      | 3.2   | 3.15   |
| Soya oil                  | 7.8   | 13.5   |
| Premix                    | 6.8   | 6.8    |
| Lysine                    | 2.7   | 2.7    |
| Methionine                | 0.3   | 0.7    |
| Total                     | 1000  | 1000   |

Nutritional composition (g/kg)

| Ingredient                  | MOLM0  | MOLM70  |
|-----------------------------|--------|---------|
| Dry matter                  | 896.0  | 851.0   |
| Crude protein               | 189.0  | 189.0   |
| Ether extract               | 52.0   | 61.0    |
| Ash                         | 49.0   | 45.0    |
| Acid detergent fibre        | 36.0   | 47.0    |
| Neutral detergent fibre     | 96.0   | 106.0   |
| Crude fibre                 | 36.0   | 34.0    |
| Metabolisable energy (KCal/kg) | 3157.6 | 3157.2 |
| Lysine                      | 9.7    | 9.7     |
| Methionine                  | 4.0    | 4.3     |

*Diet*: MOLM0 = broiler finisher without MOLM inclusion; MOLM70 = broiler finisher diluted with 70 g MOLM/kg; MOLM = *Moringa oleifera* leaf meal.

Experiment 1B – semen and sperm quality

Forty PK roosters were raised on a starter mash for four weeks. At five weeks of age (301 ± 22.70 g), the chickens were randomly allotted to two experimental diets until 40 weeks of age. A CRD was used for this experiment with a cage holding five roosters, replicated four times. Semen was collected from five roosters for six days by means of the dorso-abdominal massage method (Burrows and Quinn 1937) with a day interval between 9:00 and 11:00. Average semen (0.39 ± 0.14 ml) was collected from individual roosters into a 15 ml tube, placed in a thermo flask at 41°C and transported to the mobile laboratory.

The motility parameters measured by computer-assisted sperm analyser (CASA) (Microptic, Spain, at a magnification of × 10 – Nikon®, China) included the following: curvilinear velocity (VCL), velocity over the actual sperm track, which included all deviations of sperm head movement; average path velocity (VAP), velocity over a calculated, smoothed path; straight-line velocity (VSL), velocity over the straight-line distance between the beginning and end of the sperm track; and linearity (LIN) (see Figure 1). Two hundred microlitres of swim-up media (Kobidiol + extender) placed Eppendorf tube and added 10 µl of semen. Five microlitres of the solution was then used for CASA. A total of five fields were captured and saved for further analyses. Semen pH was measured using a calibrated pH metre (Hanna instruments®, Portugal). Twenty microlitres of ecosin stain and 7 µl of semen were mixed into the append of the tube and 5 µl mixture was used for morphology evaluation using a fluorescence microscope. An average of 300 sperms per slide per replica per cock was used for morphology assessment (Bakst et al. 1994). Undiluted 20 µl of semen was placed into microcuvettes and placed into the spectrophotometer (spermacue) for semen concentration. The sperm output for each cockerel was calculated as the product of

![Figure 1. Effects of replacing soybean meal in chicken diets with graded levels of MOLM on mean weekly feed intake of PK hens from Weeks 1–12 (total of 20 hens/treatment).](image-url)
semen volume (ml) and sperm concentration (10^9/ml) (Anderson 2001; Cerolini et al. 2006) and data were analysed using Model 1.

**Experiment 1C – fertility, hatchability and chick weight**

At Week 31, a mating ratio of 5 (hens experiment A) to 1 (rooster experiment B) was housed in an eight deep litter pen. They were offered diets shown in Table 1 for five weeks: the first week was for adaptation and egg collection was done for four weeks. Egg collection was done for three days and stored at 18°C prior to setting. Fifty eggs per treatment were randomly selected, sprayed with disinfectant and placed in an incubator with the broad end pointing upwards set at a temperature of 37.5°C and relative humidity (RH) of 65% for 18 days. Candling was done on the 7th and 18th day of incubation, and the fertile eggs only were then transferred into a hatchery at a temperature of 37.4°C and RH 70% until hatching. After hatching, the chicks were counted and the eggs that were not hatched were counted to calculate the fertility rate following the formula of Ashour et al. (2020).

\[
\text{Fertility} = \frac{\text{number of fertile eggs}}{\text{number of eggs}} \times 100
\]

Hatchability was expressed according to the chicks that hatched from fertile eggs using the formula of Sahin et al. (2009).

\[
\text{Hatchability} = \frac{\text{number of chicks hatched}}{\text{number of fertile eggs}} \times 100
\]

The hatched chicks were weighed using a digital weighing scale (Explorer EX224, 0.01 g readability (two decimal places) OHAUS Corp, Parsippany, NJ, USA) and the data collected were analysed following Model 1.

**Statistical analyses**

Data on egg hatchability, fertility rate, hatching of fertile eggs and weight of day-old chicks were analysed using the GLM procedure of SAS (2010), with diet as the only effect (Model 1). Data on feed intake, weight gain, feed conversion ratio and semen volume and characteristics were measured on a weekly basis and were analysed using the fixed model procedure of SAS (2010), which took the effects of both diet and week of measurement (Model 2) into account.

The statistical models employed were as follows: Model

\[ Y_{ij} = \mu + d_i + e_{ij}, \]  

where \( Y_{ij} \) = response variable, \( \mu \) = general mean, \( d_i \) = the fixed effects of diets, \( e_{ij} \) = random error associated with observation, \( ij \) = assumed to be normally and independently distributed.

Model

\[ Y_{ij} = \mu + d_i + w_j + (d \times w)_{ij} + e_{ijk}, \]

where \( Y_{ij} \) = response variable, \( \mu \) = general mean, \( d_i \) = effects of diets, \( w_j \) = effects of time, \( d \times w \) = effects of time interacting with diets and \( e_{ijk} \) = random error term.

For all the statistical tests, least square means was compared using the probability of difference (PDFF) option in the LSMEANs statement of SAS. The level of significance was set at \( P < .05 \).

**Results**

**Experiment 1A – growth performance**

There was no significant dietary effect on the overall weekly feed intake of the PK hens. However, the feed intake was lower in hens fed the MOLM0 diet in all the weeks except in Weeks 1 and 8 compared to those fed the MOLM70 diet (Figure 1). Feed intake decreased at Weeks 7 and 11 in hens fed MOLM0; for hens fed MOLM70, feed intake decreased in Weeks 7, 8 and 10. Diet had no significant effect on the weight gain of PK hens (Figure 2). However, there was no indication of weight loss in this study. There was no dietary effect (\( P > .05 \)) on the FCR of the PK hens (Figure 3). However, a linear increase in the FCR was observed throughout the experiment for hens fed all the diets.

**Experiment 1B – semen and sperm quality**

The statistical differences in the effects of diet and day and their interaction on semen characteristics are presented in Table 2. Dietary treatment had an effect (\( P < .05 \)) on semen pH but...
Semen characteristics & Treatment & Day & Interaction

| Parameters       | Diet (g/kg) | MOLM0 | MOLM70 | Stdev |
|------------------|-------------|-------|--------|-------|
| Semen traits     |             |       |        |       |
| Concentration (10⁸ cells ml⁻¹) | 677.50      | 719.19 | 161.13 |
| pH               | 6.94b       | 7.37a | 0.56   |
| Volume (ml)      | 0.39        | 0.45  | 0.27   |
| Progression (%)  |             |       |        |       |
| Total motility   |             |       |        |       |
| PM               | 39.38a      | 57.73a| 10.54  |
| NPM              | 48.59a      | 39.89b| 5.57   |
| Static           | 12.57a      | 2.38b| 9.40   |
| Velocity (%)     |             |       |        |       |
| Rapid            | 81.27b      | 89.06a| 10.88  |
| Medium           | 55.84       | 56.42 | 3.21   |
| Slow             | 32.78       | 32.86 | 3.24   |
| Motility         |             |       |        |       |
| VCL (µm/s)       | 86.70b      | 106.64a| 34.89 |
| VSL (µm/s)       | 34.42a      | 45.54a| 14.69  |
| VAP (µm/s)       | 51.57b      | 66.19a| 21.74  |
| Linearity (%)    | 39.93       | 42.70 | 5.07   |
| Straightness (%) | 67.08       | 68.83 | 4.61   |
| Wobble (%)       | 59.29       | 61.89 | 4.23   |
| Morphology       |             |       |        |       |
| Live             | 80.08       | 80.78 | 8.48   |
| Tail             | 4.67        | 3.67  | 2.47   |
| Dead             | 12.86       | 13.47 | 7.06   |
| Mid              | 2.17        | 1.67  | 1.62   |

Notes: *b* means within the same row with different superscripts differ (*P < .05*). VCL = curvilinear velocity; VAP = average path velocity; VSL = straight-line velocity; LIN = linearity; MOLM0 = basal diet without MOLM inclusion; MOLM70 = basal diet diluted with 70 g MOLM/kg. Stdev = standard deviation.

Table 2. Effects of replacing soybean meal in chicken diets with graded levels of MOLM on semen characteristics and quality (mean ± Stdev).

Table 3. Effects of replacing soybean meal in chicken diets with graded levels of MOLM on semen characteristics and quality (mean ± Stdev).

The observation that the inclusion of MOLM in PK hens’ diets had no significant effects on feed intake, weight gain and FCR contrasts with those reported for broilers when MOLM was included (Safa and Tazi 2012). The differences observed might be caused by the chicken breed used. The general increase in body weight of PK hens indicates that the treatment had no adverse effect on the growth and body weight of the hens. Vast literatures (Sebola et al. 2015; Olugbemi et al. 2010) reported similar results with the current results on feed intake and FCR when MOLM is included in the diets of chickens. It is known that MOLM contains bioactive compounds (Mbikay 2012). The synergy between each bioactive compounds in MOLM may be an important feature of their action which may affect nutrient absorption and processing, red-ox state, or immunity.

Discussion

**Experiment 1A – growth performance**

Bearden et al. (2004) mention that low sperm concentration has been associated with low fertility. Therefore, the higher sperm concentrations recorded for MOLM70 suggest that testicular development and proper hormone balance in PK roosters were triggered by the inclusion of MOLM. This is supported by the findings of Saalu et al. (2011) who reported that rats fed MOLM showed high sperm production and normal seminiferous epithelium. The non-significant finding of sperm concentration in this study agrees with the report by Olubowale et al. (2014), who recorded a non-significant sperm concentration when dietary lipid sources were included in the diet of Hy-line silver-brown cockerels. However, the study differs from the findings of some authors (Cerolini et al. 2006; Yusuf and Almahofid 2014) who reported a significant effect on sperm concentration when incorporating fish oil and dietary *M. oleifera* leaf meal in the diet of turkey roosters.

The pH results obtained with MOLM70 were within the range obtained by other studies in the literature (Gebriel et al. 2009; Ogbe and Affiku 2012; Orunmuyi et al. 2013). Improved mean pH (7.37) in PK roosters offered MOLM70 in this study improved sperm velocity by increasing the pH to alkaline. The literature (Ashizawa and Wishart 1987; Ashizawa et al. 1997) states that the percentage of motile sperm and sperm velocity was increased at alkaline pH in domestic roosters. Lower pH is detrimental to spermatogenesis as shown by the lower metabolic production of lactic acid in active sperm.

The significant differences reported for progressive motility in the present study highlighted the positive effect of MOLM in

Table 2. Effects of replacing soybean meal in chicken diets with graded levels of MOLM on treatment, day and their interaction (treatment × day) effect on semen quality.

Table 3. Effects of replacing soybean meal in chicken diets with graded levels of MOLM on semen characteristics and quality (mean ± Stdev).
enhancing sperm motility by providing the substrate (ATP) needed for motility. The increased progressive motility from PK roosters fed MOLM70 may be attributed to vitamin E (Sebola et al. 2017; Fuglie 2013) and selenium found in MOLM. Reports (Young et al. 1986; Hansen and Deguchi 1996) state that dietary selenium causes an increase in sperm concentration, sperm motility and sperm capacity in farm animals (including poultry species).

Plant material like MOLM contains substantial amounts of beneficial antioxidants, phytochemicals, minerals and vitamins known to increase growth and stimulate reproduction in animals, including poultry species (Mahfuz and Piao 2019).

An indicator of some disorders in spermatogenesis is sperm morphology. The MOLM contains essential antioxidant and phenolic compounds that help in protecting the testes against morphologic, spermatogenic and oxidative changes brought on by toxic materials and certain anti-neoplastic agents (Saalu et al. 2011; Orunmuyi et al. 2013). However, in the present study, dietary treatment had no significant effect on sperm morphology. But this was not the case with the report of Yusuf (2014), who noted a significant effect on the sperm morphology of turkey fed *M. oleifera* and *Gongronema latifolium* leaf meals.

**Experiment 1C – fertility, hatchability and chick weight**

The most sensitive parameters to genetic and environmental influences are fertility and hatchability (Peters et al. 2008). Reports (Stahl et al. 1986; Peebles and Brake 1987) state that factors affecting hatchability and fertility include bird strain, plane of nutrition, condition and length of storage of eggs, egg quality and mating ratio. Fertility and hatchability of fertile eggs were actually improved in chickens fed MOLM70, compared with those on MOLM0. moyo et al. (2011) showed that MOLM contains high levels of zinc and vitamin E, but Park et al. (2004) and Amen and Al-Daraji (2011) reported that zinc and vitamin E could play a beneficial role in the hatchability of eggs. Zinc helps with the protection of genetic material structure or deoxyribonucleic acid (DNA) chromatin in the sperm nucleus, which is an important structure for successful fertility. Durmus et al. (2004) noted increased hatchability with increasing zinc in the diets of brown parent stock layers.

Organically bounded selenium supplementation of laying hens’ diets improved the environment of the sperm storage tubules in the hen’s oviduct, increasing the interval of time the sperms can be stored, allowing the sperms to live longer and increasing the number of sperm holes in the yolk layer (Agate et al. 2000; Davtyan et al. 2006; Hanafy et al. 2009). Osman et al. (2010) reported that supplementation of plant leaves containing selenium increased fertility and hatchability percentage. Narushin and Michael (2002) state that the physical characteristics of the egg such as the shape index, weight, length and width play a significant role in embryo development and successful hatching. This might be the reasons for improved fertility and hatchability in the group fed MOLM70 in the present study. The hatchability of fertile eggs noted in
In conclusion, the findings of the current study indicate that the inclusion of MOLM significantly improved semen quality parameters such as the progression, velocity and motility of sperm cells. Also, feeding MOLM improved subsequent hatchability traits and chick weight.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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