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Effect of Dietary Supplementation of *Moringa oleifera* Leaf Meal on Production and Reproduction in Rhode Island Red Chicken

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ABSTRACT

A study was carried out to determine the effect of *Moringa oleifera* leaf meal (MOLM) on production performance, semen quality, fertility, hatchability and chick quality of Rhode Island Red (RIR) chickens. One hundred and eighty, 24-week-old Rhode Island Red layer birds were randomly allotted to four dietary treatments having replicates with 15 birds in each. The treatments were T1 (Control: Basal diet); T2: 1% MOLM in basal diet; T3: 3% MOLM in basal diet; T4: 5% MOLM in basal diet. To study reproductive performance, 24 cocks at the age of 24th weeks were kept separately and equally divided into four groups and were provided same dietary treatments as provided to hens. The results revealed that average daily feed intake per bird (g/day) was lower ($P<0.05$) for hens supplemented with MOLM. No statistical difference for feed conversion ratio (FCR) was observed between groups. The average HDEP % and HHEP % of experiment were significantly ($P<0.05$) higher in T3 and T4 groups as compared to T1 The MOLM diet resulted in significantly ($P<0.05$) increased semen concentration, total sperm count, motility and live sperm than the control, however semen volume and pH were not differed between groups. Fertility % was linearly increased with MOLM but it was not statistically different ($P>0.05$) with control group. Hatchability percentage was higher ($P>0.05$) in T3 and T4 than the others. Hens in T2, T3 and T4 groups had higher mean values for average chick weight and chick length ($P<0.05$) than the control group. Results of the present study indicated positive response of *Moringa oleifera* leaf meal on production and reproduction performance when used in the diet of birds.

KEY WORDS: Fertility, Moringa leaf meal, Rhode Island Red, Semen

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INTRODUCTION

In intensive poultry farming, feed remain to be the biggest challenge, particularly in India where the cost of traditional feed supplies is constantly rising. The scarcity and ensuing high cost of conventional protein sources, which are limiting constraints for the manufacturing of chicken feed, had an impact on poultry productivity. Addition of antibiotic growth promoters to feed, which increased feed productivity, efficiency and financial gain, has been banned from poultry feed (Fallah et al., 2013). Dietary manipulation has been recommended as a method of improving reproduction due to the strong relationship between nutrition and overall fertility (Hudson and Wilson 2013). Peters et al. (2008) reported semen quality as the measure of the capacity of semen to achieve fertilization, which is essential in the production of hatching eggs and can be heavily influenced by nutrition.

One such alternative is phytobiotics, which are defined by Windisch et al. (2008) as plant-derived products added to feed to enhance the performance of farm animals. Tree leaves are a good source of vitamins, vital amino acids, proteins and minerals and have a wide range of nutrients (Fasuyi, 2006). Among the vital elements found in *Moringa oleifera* leaves, protein, vitamin B complexes, vitamin C, beta-carotene, vitamin K and manganese are the most nutrient-dense components (Leone et al., 2015). There is minimal data on the use of *Moringa oleifera* as a protein source in the layer ration for poultry feeding, despite its high nutritional content. Therefore, this study was conducted to determine effects of feeding MOLM on production and reproduction performance in laying hens.

MATERIALS AND METHODS

The experiment was conducted at the Livestock Farm Complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh Gujarat (latitude 21°29' N, longitude 70°26' E and altitude 60 meters above the mean sea level). Laboratory work was carried out in the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh. The climate in Junagadh is tropical and arid.

Experimental details

Rhode Island Red layer birds (n=180) of 24 weeks of age were selected at random and divided into four equal groups of 45 birds with three replicates of 15 in each group in a completely randomized design. The birds were raised in cages under uniform management and fed the respective diets from 24th to 40th weeks of age. To study reproductive performance, 24 cocks at the age of 24th weeks were kept separately and equally divided into four groups and were provided same dietary treatments as provided to hens. Research protocol was approved by the Animal Ethics Committee, vide protocol no.: KU-JVC-IAEC-SA-96-2022.

Four experimental diets (isocaloric and isonitrogenous) were prepared with MOLM as T1 (Control: Basal diet); T2: 1% MOLM in basal diet; T3: 3% MOLM in basal diet; T4: 5% MOLM in basal diet (Table 1). Feed was offered ad libitum in weighed quantity twice a day at 9:00 AM and 5:00 PM. Manual turning and mixing of feeds in the feeder were done frequently at least twice daily. Clean, fresh, wholesome drinking water was provided to all experimental birds *ad libitum*. Light bulbs were placed for the lighting system to increase the lighting period to 16 h per day.

Table 1. Ingredient and nutrient composition of the experimental diets

Ingredients (%)	T1	T2	T3	T4
Ingredient composition (% DM basis)				
Maize	53.4	53.4	50	45
Soyabean DOC	27.7	26.7	24.7	22.7
Deoiled Rice Bran	6.41	6.41	9.81	14.8
Moringa oleifera leaf meal (MOLM)	0	1.0	3.0	5.0
Calcite Powder	3.0	3.0	3.0	3.0
Limestone	6.9	6.9	6.9	6.9
DCP	1.63	1.63	1.63	1.63
Salt	0.3	0.3	0.3	0.3
Premix (Vitamins, Enzymes etc.)	0.66	0.66	0.66	0.66
Total %	100	100	100	100
Calculated nutrient composition (%)				
ME (Kcal/kg)	2613	2616	2622	2629
DM	89.5	89.3	89.3	89.8
OM	93.0	93.0	92.9	92.8
CP	18.0	18.4	18.6	18.3
CF	8.17	8.01	7.92	7.94
EE	3.45	3.51	3.67	3.8
NFE	63.4	63.0	62.6	62.7
Total Ash	6.92	6.95	7.1	7.2
Silica	1.11	1.22	1.16	1.19
Calcium	3.05	3.21	3.28	3.36
Phosphorus	0.38	0.39	0.39	0.4

Feed intake, feed conversion ratio and egg production

Feed consumption of each replicate was recorded daily. The amount of feed consumed per bird was determined as the difference between the feed offered and left over to calculate weekly/daily feed intake (g). Feed conversion ratio was determined weekly as a unit feed consumed per unit egg weight (Abou-Elezz et al., 2011).

Eggs were collected two times a day at 9:00 and 17:00 hours. The sum of the two collections along with the number of birds alive on each day was recorded and summarized at the end of the period. Hen-day egg production (HDEP) and hen housed egg production (HHEP) as percentage were determined. Eggs were weighed weekly immediately after collection and average egg weight was computed by dividing the total egg weight to the number of eggs.

Semen quality, fertility and hatchability of eggs and chick quality

At the end of experiment, semen quality was evaluated in 24 cocks (6 cocks in each treatment). Before the commencement of semen sampling, the cocks were trained for a period of four weeks for semen collection. Using an abdominal massage technique, semen was collected from every cock in each replicate and examined for semen quality traits (Lake, 1957). The cloacal region was massaged to induce phallic tumescence; this was followed by a cloacal stroke and a squeeze of the area around the edges of the cloaca to express the semen. Semen was milked down into graduated collecting glass test tubes and its volume was then measured. Each cock's fresh semen samples was measured using a pH strip by dropping the sample on the strip and reading the pH. Semen was diluted to 1:4 with phosphate buffered saline, mixed, and incubated at 37°C to test for individual motility. A cover slip was placed over a drop of diluted semen on a warm slide, and a light binocular microscope set to 400X magnification was used to study it. A thin smear was created by mixing 10 ìl of diluted semen with 10 ìl of eosin-nigrosin stain at 37°C in order to determine the viability. Using a hemocytometer, the sperm cell concentrations were calculated and expressed in billions/ml of semen. Sperm count was done as described by Hafez (1987) with a light microscope (400X).

At the end of experiment, fertility was assessed by performing artificial insemination with standard protocol in experimental birds by semen collected from respective cocks. Fifteen 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 7^{th} and 18^{th} 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. Average percentage fertility was determined by dividing the total number of eggs found fertile at candling by total number of eggs set times 100. Average percentage hatchability of the fertile eggs were computed by dividing the number of chicks hatched by the number of fertile eggs times 100.

Chick quality assessment was performed by employing chick weight and chick length at hatching. Chick length was determined by stretching the chick along a ruler and measuring the length from beak to the end of the middle toe. Chick weight was measured by weighing the chick at hatching.

RESULTS AND DISCUSSION

Production performance

In the current study, it was found that overall average feed intake per bird (g/day) was higher (P<0.05) for hens in T1 than hens in T2, T3 and T4 (Table 2). Hens in T2 had higher (P<0.05) average feed intake per bird (g/day) than hens in T4, however, T3 did not significantly differ (P>0.05) with T2 and T4. This was similar to the findings of Raphael et al. (2015) noted that addition of 5% and 10% MOLM to the laying hen diet reduced feed consumption. They attributed the significant decline in feed intake with increasing amount of leaf meal due to its high fibre content as well bitter taste. In contrast, Sharmin et al. (2021) observed that average daily feed consumption of laying hens fed diets containing 1.5% MOLM was significantly higher than that of the hens fed diet with 0%, 0.5% and 1% MOLM.

Table 2. Effects of dietary treatment on production parameters of RIR laying hen

Parameter	T1	T2	T3	T4	P value	SEM
Feed Intake, g/day	116± 0.44^{A}	114± 0.36^{B}	112± 0.55^{BC}	111± 0.85^{C}	0.001	3.1
Feed Conversion Ratio (FCR)	2.94±0.03	2.86±0.03	2.93±0.01	2.91±0.05	0.96	0.25
HDEP %	69.08±1.88^{B}	73.76±1.5^{AB}	75.67±1.71^{A}	79.17±1.15^{A}	0.002	4.97
HHEP %	65.96±2.11^{C}	72.13±1.79^{BC}	74.44±1.91^{AB}	78.74±1.28^{A}	0.003	5.83

Means bearing superscripts in the same row differ significantly (P<0.05). SEM, Standard error of mean; P, Probability

In the current study, no statistical difference was observed for FCR between groups or within group. Overall average FCR was lowest for hens in T_2. But, there was no statistical differences (P>0.05) among the groups. The results are similar with the finding of Olugbemi et al. (2010) who noted that addition of 5% and 10% MOLM to the laying hen diet had no effect on feed conversion ratio. However, Raphael et al. (2015) gradually replaced soybean with MOLM at 0, 5 and 10% and observed that with 5% MOLM, the FCR was lower; however, with 10% MOLM, the FCR was higher.

The values were significantly (P<0.05) higher in T_3 and T_4 groups as compared to T1 group and numerically higher than T2 group. The values were gradually increased from T1 to T4 and have significantly higher values in T3 and T4 as compared to control. The T3 was non-significant different from T2 and T4. T4 also showed significantly higher value than T2. In the present investigation, an increase in laying percentages with advancement of age was noted in all dietary treatments. Current study was in line with Ebenebe et al. (2013) who reported similar results in response to various levels (0%, 2.5%, 5.0% and 7.5%) of MOLM in diets of laying chickens. Raphael et al. (2015) also noted significant effect on egg production when fed 5% MOLM as compared to 0 and 10% MOLM in Kabir strain chickens. On the other hand, Olugbemi et al. (2010) showed a non-significant effect on laying % for hens fed a diet containing MOLM at 0, 5, and 10% of the diet. Abu and Akangbe (2017) also found that addition of 0, 1 and 5% MOLM Japanese quails' diet had no significant effect on HDEP % when compared to a diet free of MOLM. Improved balanced nutritional supply provided by MOLM in the diet may be the cause of the greater egg production in layers given the diet containing MOLM. Lysine, methionine, and a variety of other amino acids are present in MOLM, which may provide the necessary quantity of nutrients for improved production.

Reproduction performance

Effect of feeding MOLM at different levels on semen characteristics in Rhode Island Red cocks indicated that dietary treatment had significant effect (P<0.05) on sperm motility, live and dead sperm, sperm concentration and total sperm count but did not affect semen volume and pH. Sperm concentration was significantly higher in all level MOLM supplemented groups and higher value was found in 3% MOLM group (Table 3). Total sperm count was also significantly higher in treatment groups, which might be attributed to the increased semen volume and sperm concentration. Sperm motility was recorded to be higher in 5% MOLM supplemented group and it was significantly (P<0.05) higher than control. Live and dead sperm % were also significantly affected with MOLM supplementation.

Table 3. Effects of feeding different levels of MOLM on semen quality parameters of Rhode Island Red cocks

Parameters	T1	T2	T3	T4	SEM	P value
Body Weight (g)	2718 ±85.59	2683 ±134.85	2655.75 ±33.88	2662.75 ±230.98	282	0.9892
Volume (ml)	0.34 ±0.06	0.39 ±0.04	0.46 ±0.04	0.38 ±0.05	0.1	0.3979
pH	7.5 ±0	7.63 ±0.12	7.63 ±0.13	7.63 ±0.13	0.21	0.8015
Concentration ($x10^9$/ml)	1.44 ±0.11[B]	2.41 ±0.19[A]	2.49 ±0.19[A]	2.34 ±0.24[A]	0.38	0.0001
Total sperm count ($x10^9$/ejaculate)	0.49 ±0.08[B]	0.93 ±0.15[A]	1.14 ±0.18[A]	0.88 ±0.16[A]	0.3	0.006
Motility (%)	73.75 ±6.25[B]	81.5 ±0.95[AB]	86.75 ±2.35[AB]	88.25 ±1.18[A]	6.8	0.0443
Live sperm (%)	75.75 ±2.52[B]	85.25 ±1.65[A]	85.5 ±2.21[A]	85.25 ±2.05[A]	4.27	0.0174
Dead sperm (%)	24.25 ±2.52[A]	14.75 ±1.65[B]	14.5 ±2.21[B]	14.75 ±2.05[B]	4.27	0.0174

Means bearing superscripts in the same row differ significantly (P<0.05). SEM, Standard error of mean; P, Probability.

Sebola et al. (2022) found that addition of MOLM at 70 g/kg diet caused increased sperm motility and elevated (P<0.05) semen pH in Potchefstroom Koekoek (PK) chickens. However, the PK cockerels' semen volume was unaffected by diet (P>0.05). Poku et al. (2023) observed that the dietary MOLM had significant influence (P<0.05) on sperm motility, semen pH and sperm count in Pearl Guinea fowl cock. Increasing levels of MOLM in the diet decreased sperm motility (P<0.05).

Optimal qualities in cock semen are necessary for increased reproductive effectiveness. In this study, dietary MOLM resulted in greater semen pH, sperm count, and decreased dead sperm. Physiologically, higher semen pH, sperm count and low dead sperm obtained with dietary MOLM indicated that increasing MOLM in the diet increased the levels of essential amino acids such as lysine, phenylalanine, valine, histidine and isoleucine. The superior semen quality shown in Rhode Island Red cocks fed MOLM might be due to the high concentrations of healthy antioxidants, phytochemicals, minerals, and vitamins found in MOLM.

Fertility % linearly increased with MOLM but it was not statistically different (P>0.05) with control group. Hatchability percentage was higher (P>0.05) in T3 and T4 than the others (Table 4). Hens in T2, T3 and T4 groups had higher mean values for average chick weight and chick length (P<0.05) than the control group.

Table 4. Fertility, hatchability and chick quality of Rhode Island Red hens fed different levels of MOLM

Parameters	T1	T2	T3	T4	X^2 Value	Df	P Value
Fertility (%)	66.67 ±6.66	86.67 ±6.66	93.33 ±6.66	93.33 ±6.66	5.621	3	0.165
Hatchability (%)	61.11 ±5.55	61.67 ±7.26	65.0 ±5.0	65.0 ±5.0	1.607	3	0.769
Parameters	T_1	T_2	T_3	T_4	SEM		P Value
Chick Weight (g)	37.46 ±0.22[B]	41.68 ±0.34[A]	41.61 ±0.1[A]	41.5 ±0.54[A]	1.11		0.0001
Chick length (cm)	15.92 ±0.25[B]	17.34 ±0.13[A]	17.19 ±0.1[A]	17.3 ±0.21[A]	0.49		0.0001

Means bearing superscripts in the same row differ significantly (P<0.05). SEM, Standard error of mean; P, Probability

Result obtained in the current study were consistent with the finding of Raphael et al. (2015) using *Moringa oleifera* leaf meal as an alternative feed ingredient in the Kabir strain hens' ration which showed non-significant (P>0.05) effect of MOLM on fertility and hatchability. Similarly, Ashour et al. (2020) found that dietary MOLM had no negative effects on fertility and hatchability in Japanese quails. Contrary to present findings, Alebachew et al. (2016) observed that fertility and hatchability were significantly improved by feeding of MOLM in dual-purpose Koekoek hens. However, average chick weight and chick length were significantly higher (P<0.05) in MOLM supplemented groups. Mousa et al. (2017) also concluded that diets supplemented with MOLM demonstrated significantly (P<0.05) improved fertility and hatchability.

In current study it was found that addition of MOLM in diet of hens improved fertility, hatchability and chick quality in terms of chick weight and chick length. Micro nutrients are key factor in successful poultry reproduction. These nutrients like calcium, phosphorus, zinc, iron, vitamin E, vitamin C etc. are relatively high in MOLM. This result may be due to the fact that herbal plant may provide some compounds that enhance egg quality and fertility.

CONCLUSION

This study concludes that addition of *Moringa oleifera* leaf meal up to 5 % level in the diet of Rhode Island Red laying hens improved production performance and reproduction efficiency.

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