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## Effect of using *Moringa oleifera* (Drumstick) leaf meal on performance of Japanese quail

A Talukdar, D Choudhury, KP Kalita and R Saikia

**Abstract**

The study aimed to investigate the effects of feeding different levels of *Moringa oleifera* (Drumstick) leaf meal on the performance of Japanese quail (*Coturnix coturnix japonica*). A total of 300 day-old commercial Japanese quail chicks from a single hatch was taken for the 6 weeks trial and divided into five groups (60 birds per group). The birds were fed with different levels of *Moringa oleifera* leaf meal and were studied for various performance parameters of Japanese quail. Result showed significant increase in body weight, body weight gain, improved FCR and increased gross profit for Japanese quail fed with *Moringa oleifera* leaf meal compared to the control group without any supplementation. *Moringa oleifera* leaf meal can be used economically as a natural feed additive in Japanese quail diet at the level of 0.25 to 0.50% to improve the overall performance of Japanese quail.

**Keywords:** Drumstick, feed additives, *Moringa oleifera*, performance, phytogetic

**1. Introduction**

Poultry industry is a predominant source of animal protein in both developed and developing countries. In an attempt to augment the animal protein for human consumption quails were introduced. They are highly prolific with short generation interval and are less susceptible to diseases. They therefore facilitated to bridge the gap for other animal products to be available on the table for human consumption. With the present trends of rising price, poultry production sectors in developing countries are facing some threats, one of which is increase in the cost of feed due to high prices of protein and energy sources (Abbas 2013; Moreki and Gabanakgosi, 2014) [1, 23]. In addition, problems have aroused as a result of development of antibiotic-resistant pathogens due to unwise and excessive use of antibiotics. Poultry production plays an important socio-economic roles in developing countries because poultry are important and cheap source of animal protein (Olwande *et al.* 2010 and Melesse *et al.* 2013) [27, 22]. The purpose of modern poultry production systems is to obtain maximum profit at minimum production cost, and 60- 70% of this production cost consists of the feed cost (Tesfaye *et al.* 2013) [34]. High cost and scarcity of ingredients has raised the need for certain cheap, locally available and less competitive substitutes to be used as ingredients in poultry ration (Gadzirayi *et al.* 2012) [11]. In this context, one of the alternative cheap source that can be used in poultry nutrition are the leaves of tropical legumes such as *Moringa oleifera* (Makkar and Becker, 1997; Olugbemi *et al.* 2010; Abou- Elezz *et al.* 2011; Melesse *et al.* 2013; Tesfaye *et al.* 2013) [20, 26, 3, 22, 34]. *Moringa oleifera* which belongs to family Moringaceae is highly valued plant, distributed in the tropical and subtropical areas around the world (Makkar and Becker, 1997; Khalafalla *et al.* 2010; David *et al.* 2012; Mbikay, 2012) [20, 17, 8, 21]. All parts of the *Moringa oleifera* tree, from the roots to the leaves have nutritional and pharmacological properties. Especially, the leaves of *Moringa oleifera* are highly nutritious, being a good source of protein,  $\beta$ - carotene, vitamins A, B, C and E, nicotinic acid, folic acid, pyridoxine, amino acids, minerals and various phenolics compounds (Abbas, 2013; Anwar *et al.* 2007; Verma *et al.* 2009; Amaglo *et al.* 2010; Mbikay, 2012; Moyo *et al.* 2012a) [1, 6, 37, 5, 21, 24].

The *Moringa oleifera* plants are used culturally for their nutritional, medicinal properties as well as taste and flavour. The leaves of *Moringa oleifera* can be consumed fresh, boiled, or kept as a dried powder without any major damage. The presence of vitamin C, vitamin E, carotenoids, flavonoids and selenium make *Moringa oleifera* a potential antioxidant (Moyo *et al.* 2012a) [24]. *Moringa oleifera* have been reported to improve shelf-life and the quality of meat products (Valeria and Williams, 2012) [36].

It seems to reduce the activity of pathogenic bacteria and molds and improves the digestibility of other foods, thus helping chickens to express their natural genetic potential (Gaia, 2005) [12]. In the recent years, there is a demand for natural foods that contain active substances such as phytochemicals (Godinez- Oviedo *et al.* 2016) [13]. Further the different parts of *Moringa oleifera* are good sources of various phytochemicals such as alkaloids, flavonoids, phenolics, glucosinolates, carotenoids, sterols (Anwar *et al.* 2007, Verma *et al.* 2009, Mbikay, 2012, Padayachee and Bajinath 2012, Umsha *et al.* 2013) [6, 37, 21, 28, 35]. These phytochemicals gives healing properties to *Moringa oleifera* (Padayachee and Bajinath 2012, Gopalakrishnan *et al.* 2016) [28, 14]. It is also reported that *Moringa oleifera* has many positive effects like anti-oxidant (Vongsak *et al.* 2013) [38], anti-tumor (Khalafalla *et al.* 2010) [17], anti-inflammatory (Rajanandh *et al.* 2012) [30], anti-diabetic (Jaiswal *et al.* 2009) [16], anti-bacterial (Moyo *et al.* 2012b) [25], hypolipidemic (Sangkitikomol *et al.* 2014) [32], immunomodulatory (Sudha *et al.* 2010) [33], hepatoprotective (Al-Said *et al.* 2012) [4]. Considering the above facts in view, the present study was undertaken with the objective to study the effects of feeding different levels of *Moringa oleifera* (Drumstick) leaf meal on the performance of Japanese quail (*Coturnix coturnix japonica*).

## Materials and Methods

The study was undertaken at the Instructional Poultry Farm, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India. For the present study 300 numbers of Japanese quail chicks having similar body weight from a single hatch was procured and randomly divided into 5 groups viz, T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> with 60 number of chicks in each groups, which was further divided into 3 replicates of 20 chicks in each subgroups. The chicks were wing banded, weighed and reared under cage system of rearing. The birds under T<sub>0</sub> group (control) were offered basal diet without addition of *Moringa oleifera* leaf meal. The birds under T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> groups were fed basal diet supplemented with *Moringa oleifera* leaf meal at the rate of 0.25, 0.50, 0.75 and 1.00% in the feed (on dry matter basis), respectively. For preparation of *Moringa oleifera* leaf meal, raw *Moringa oleifera* leaves were collected from local source. Then leaves were sun dried for 10 days. The dried *Moringa oleifera* leaves were pulverized and stored at room temperature and used in the quail starter and finisher feed for a period of 6 weeks (Fig 1(a), 1(b), 1(c) & 1(d)). All the birds under the control and treatment groups were offered *ad libitum* feed and water throughout the experimental period. The birds of all the five groups were reared separately and maintained under uniform managerial condition. Three types of diets were prepared, that is Quail starter (0-3 weeks) and Quail finisher (4-6 weeks) as per the recommendation of Indian Council of Agricultural Research (2013) containing the feed stuffs namely maize, rice polish, soya bean meal, ground nut cake, mineral mixture and common salt at recommended levels (Table 1.0). During the experimental period, data on the following parameters were recorded and analysed such as weekly feed intake and total feed consumption, weekly body weight and body weight gain, Feed Conversion Ratio (FCR), livability and economics of production using Complete randomized design (CRD) and One way Analysis of Variance performed by IBM SPSS statistic 20.

**Table 1:** Ingredients and Nutrient Composition of Basal Diet (Quail Starter, Finisher and Layer Ration) As Per Icar (2013)

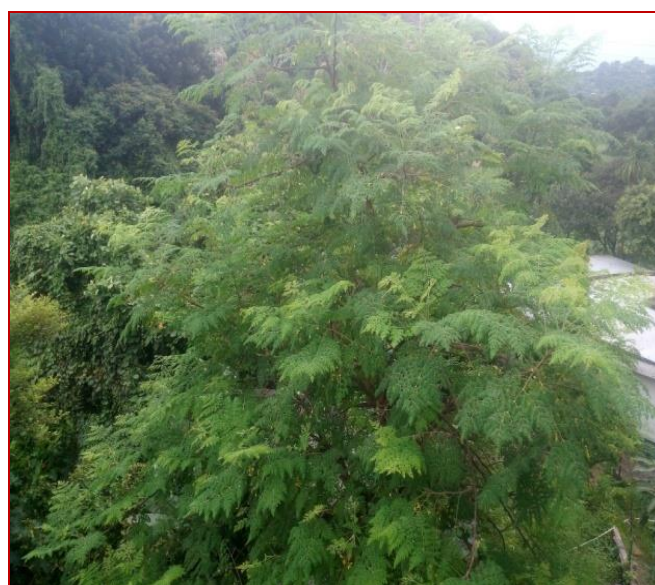
Ingredient	Starter 0-3weeks	Finisher 4-6 Weeks	Layer 7-14weeks
Maize	33.00	43.00	42.00
Rice Polish	6.35	6.30	8.40
Ground nut cake	7.15	4.00	4.00
Soya bean meal	43.00	33.50	30.00
Wheat Bran	7.00	6.20	7.10
Vegetable oil	1.00	4.50	3.00
Mineral mixture	2.00	2.00	2.00
Common salt	0.50	0.50	0.50
Shell grit	-	-	3.00
Total	100	100	100
Nutrient Composition			
Crude protein (%)	25.10	22.02	18.07
Metabolizable energy (kcal/kg) (calculated values)	2808.09	2929.49	2850.96

(N.B. Vitamin premix Provita M was added @ 20g per 100 parts of ration in all the three diet.)

**Table 2:** Composition of Provita M

Ingredients	Amounts
Vitamin A	6000 IU
Vitamin D <sub>3</sub>	2500 IU
Vitamin E	1.5 IU
Thiamine	0.2 mg
Riboflavin	1 mg
Pantothenic Acid	3 mg
Niacin	10 mg
Pyrodoxine	0.5 mg
Folic acid	0.2 mg
Vitamin B <sub>12</sub>	10 mcg
Choline	5mg
Lactic Acid Bacteria	100 million CFU

*Lactobacillus acidophilus*, *Enterococcus faecium*, *Lactobacillus plantarum*, *Lactobacillus casei*



**Fig 1(a):** *Moringa oleifera* TREE



**Fig 1 (b):** Collection of *Moringa oleifera* Leaf



**Fig 1(C):** Sundrying of *Moringa oleifera* LEAF



**Fig 1(d):** *Moringa oleifera* Leaf Meal

**Results and Discussion**

It is observed that the supplementation of *Moringa oleifera* leaf meal at 0.25, 0.50, 0.75 and 1.00% level improved the feed intake from second week of age and the trend continued till sixth week of age compared to control group of birds (Table 3.0). Similar findings with respect to improvement in feed intake were observed by Safa and Tazi (2014) [31] and Hassan *et al.* (2016) [15]. The findings of the

present study were contradictory to findings of Khan *et al.* (2017) [18], Castillo *et al.* (2018) [7] which showed no improvement in feed consumption between treatment and control groups. The higher feed intake might be due to beneficial effect of *Moringa oleifera* leaf on microbial environment in the gut which improved the digestion, absorption and nutrient utilization. (Hassan *et al.* 2016) [15].

**Table 3:** Mean Weekly Feed Intake (G/Bird) and Total Feed Consumption (G/Bird) Under Different Treatment Group

Groups Weeks	T <sub>0</sub> (Control)	T <sub>1</sub> (MOLM-0.25%)	T <sub>2</sub> (MOLM-0.50%)	T <sub>3</sub> (MOLM-0.75%)	T <sub>4</sub> (MOLM-1.00%)
1 <sup>st</sup>	43.03	42.33	39.33	40.55	40.67
2 <sup>nd</sup>	92.28	93.63	95.15	90.25	93.22
3 <sup>rd</sup>	127.88	117.20	128.17	127.35	119.93
4 <sup>th</sup>	121.40	158.58	168.90	166.55	155.3
5 <sup>th</sup>	194.21	190.19	196.12	196.65	186.02
6 <sup>th</sup>	240.55	229.61	241.90	244.27	230.05
	819.35	831.54	869.57	865.62	825.19

The mean weekly body weight (Table 4.0) of different treatment groups did not differ significantly ( $P>0.05$ ) during the first and second week of age. In the third, fourth and fifth week of age the results revealed significantly ( $P\leq 0.05$ ) higher body weight in the case of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> group compared to the control (T<sub>0</sub>) group. However, the T<sub>4</sub> group showed non-significant difference compared

to other groups. At sixth week of age, the body weight of Japanese quail differed significantly ( $P<0.01$ ) among the different experimental groups. The T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> group gained significantly higher body weight compared to control (T<sub>0</sub>) which might be due to supplementation of *Moringa oleifera* leaf in the diet of treatment groups. These findings are in agreement with the

observations made by David *et al.* (2012) [8], Dey and De. (2013) [9], Khan *et al.* (2017) [18] and Elkloub *et al.* (2015) [10]. They reported that supplementation of *Moringa oleifera* in basal diet improved the final body weight. Contrary to the present findings, few researchers Olugbemi *et al.* (2010) [26], Abbas and Ahmed (2012) [2], Mahmud *et al.* (2016) [19] reported that addition of different levels of *Moringa oleifera* leaf meal had no significant ( $P>0.05$ ) effect on body weight. The improvement in body weight observed in treatment groups

might be due to increase digestibility and absorption of nutrients from the gut (Dey and De, 2013) [9]. The non significant ( $P>0.05$ ) difference showed by T<sub>4</sub> group in the third, fourth and fifth week of age was might be due to higher level of inclusion (1%) of *Moringa oleifera* leaf meal compared to the other three treatment groups (0.25, 0.50 and 0.75% leaf meal in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively) in the diets.

**Table 4:** Mean  $\pm$  Se Weekly Body Weight (G) Of Japanese Quail Under Different Treatment Groups

Groups Weeks	T <sub>0</sub> (Control)	T <sub>1</sub> (MOLM-0.25%)	T <sub>2</sub> (MOLM-0.50%)	T <sub>3</sub> (MOLM-0.75%)	T <sub>4</sub> (MOLM-1.00%)
1 <sup>st</sup>	29.18 <sup>a</sup> $\pm$ 0.77	29.65 <sup>a</sup> $\pm$ 2.06	30.70 <sup>a</sup> $\pm$ 0.84	28.70 <sup>a</sup> $\pm$ 0.91	31.18 <sup>a</sup> $\pm$ 0.77
2 <sup>nd</sup>	73.81 <sup>a</sup> $\pm$ 1.89	75.73 <sup>a</sup> $\pm$ 1.29	75.08 <sup>a</sup> $\pm$ 1.34	74.30 <sup>a</sup> $\pm$ 1.45	71.10 <sup>a</sup> $\pm$ 2.04
3 <sup>rd</sup>	109.35 <sup>a</sup> $\pm$ 3.07	121.40 <sup>b</sup> $\pm$ 2.10	118.42 <sup>b</sup> $\pm$ 1.75	120.60 <sup>b</sup> $\pm$ 2.14	114.85 <sup>ab</sup> $\pm$ 2.03
4 <sup>th</sup>	152.49 <sup>a</sup> $\pm$ 3.46	166.38 <sup>b</sup> $\pm$ 2.29	163.40 <sup>b</sup> $\pm$ 2.15	161.40 <sup>b</sup> $\pm$ 2.01	158.06 <sup>ab</sup> $\pm$ 2.68
5 <sup>th</sup>	189.00 <sup>a</sup> $\pm$ 3.94	212.26 <sup>b</sup> $\pm$ 2.43	207.67 <sup>b</sup> $\pm$ 1.96	203.81 <sup>b</sup> $\pm$ 1.94	198.71 <sup>ab</sup> $\pm$ 2.60
6 <sup>th</sup>	224.98 <sup>a</sup> $\pm$ 4.60	251.03 <sup>b</sup> $\pm$ 3.47	250.91 <sup>b</sup> $\pm$ 2.27	244.96 <sup>b</sup> $\pm$ 2.26	241.48 <sup>b</sup> $\pm$ 3.43

Means bearing same superscripts in a row did not differ significantly

The mean weekly body weight gain (Table 5.0) of different experimental groups showed non-significant ( $P>0.05$ ) difference in the first week of age. On the second week, the mean weekly body weight gain differ significantly ( $P<0.05$ ) among the different treatment groups. In the second week, the higher body weight was recorded in all the groups except T<sub>4</sub>. However, T<sub>1</sub> and T<sub>3</sub> group showed significantly ( $P<0.05$ ) higher body weight gain compared to T<sub>4</sub> group. During the third, fifth and sixth week of age all the experimental groups showed significantly ( $P<0.01$ ) higher body weight gain compared to T<sub>0</sub>. Whereas, no significant ( $P>0.05$ )

difference was observed in the fourth week of age in terms of weekly body weight gain. These findings are in accordance with the reports of Safa and Tazi (2014) [31], Elkloub *et al.* (2015) [10], Hassan *et al.* (2016) [15] and Mahmud *et al.* (2016) [19]. Contrary to the present findings Olugbemi *et al.* (2010) [26], Abbas and Ahmed (2012) [2] and Castillo *et al.* (2018) [7] reported no significant ( $P>0.05$ ) difference in *Moringa oleifera* supplemented groups compared to control. The higher body weight gain might be due to efficient utilization of protein in the diet.

**Table 5:** Mean  $\pm$  Se Weekly Body Weight Gain (G) Of Japanese Quail Under Different Treatment Groups

Groups Week	T <sub>0</sub> (Control)	T <sub>1</sub> (MOLM-0.25%)	T <sub>2</sub> (MOLM-0.50%)	T <sub>3</sub> (MOLM-0.75%)	T <sub>4</sub> (MOLM-1.00%)
1 <sup>st</sup>	21.51 <sup>a</sup> $\pm$ 0.81	21.98 <sup>a</sup> $\pm$ 0.83	22.91 <sup>a</sup> $\pm$ 0.50	21.91 <sup>a</sup> $\pm$ 0.64	23.48 <sup>a</sup> $\pm$ 0.89
2 <sup>nd</sup>	44.44 <sup>ab</sup> $\pm$ 1.93	46.08 <sup>a</sup> $\pm$ 1.26	44.38 <sup>ab</sup> $\pm$ 1.20	45.60 <sup>a</sup> $\pm$ 1.19	39.91 <sup>b</sup> $\pm$ 1.30
3 <sup>rd</sup>	35.54 <sup>a</sup> $\pm$ 1.51	45.66 <sup>b</sup> $\pm$ 1.36	43.33 <sup>b</sup> $\pm$ 1.86	46.30 <sup>b</sup> $\pm$ 1.38	43.75 <sup>b</sup> $\pm$ 1.34
4 <sup>th</sup>	43.13 <sup>a</sup> $\pm$ 2.60	44.98 <sup>a</sup> $\pm$ 1.97	44.52 <sup>a</sup> $\pm$ 2.04	40.98 <sup>a</sup> $\pm$ 2.19	43.21 <sup>a</sup> $\pm$ 2.44
5 <sup>th</sup>	36.50 <sup>a</sup> $\pm$ 2.37	45.88 <sup>b</sup> $\pm$ 2.03	44.27 <sup>b</sup> $\pm$ 1.92	42.40 <sup>b</sup> $\pm$ 1.89	40.65 <sup>b</sup> $\pm$ 1.79
6 <sup>th</sup>	35.98 <sup>a</sup> $\pm$ 1.80	38.76 <sup>b</sup> $\pm$ 2.18	43.23 <sup>b</sup> $\pm$ 0.97	41.15 <sup>b</sup> $\pm$ 0.95	42.76 <sup>b</sup> $\pm$ 1.65

Means bearing same superscripts in a row did not differ significantly Among the different treatment groups the mean weekly Feed Conversion Ratio (FCR) of T<sub>2</sub> group showed the best FCR value in first week (Table 6.0). In the second week T<sub>3</sub> group recorded better FCR value. In the third and fifth week of age T<sub>1</sub> group recorded better FCR value. In the fourth week improved FCR was recorded in control group. In the sixth week of age T<sub>4</sub> group showed better FCR value as compared to other groups.

The overall FCR of the entire period of experiment was best in T<sub>1</sub> (3.35) followed by T<sub>2</sub> (3.39), T<sub>4</sub> (3.42), T<sub>3</sub> (3.53) and T<sub>0</sub> (3.64) group. The feed conversion ratio of the Japanese quail improved due

to supplementation of *Moringa oleifera* leaf meal in the basal diet compared to control group. These findings are in accordance with Elkloub *et al.* (2015) [10]. On the other hand, addition of *Moringa oleifera* leaf meal in the ration did not show significant ( $P>0.05$ ) difference in Feed Conversion Ratio according to findings of Abbas and Ahmed, (2012) [2], Paguia *et al.* (2014) [29] and Khan *et al.* (2017) [18]. The better Feed Conversion Ratio can be attributed to the increase digestibility and absorption of nutrients from the gut (Elkloub *et al.*, 2015) [10] in the treatment groups fed with *Moringa oleifera* leaf meal (Abbas and Ahmed, 2012) [2].

**Table 6:** Mean Weekly Feed Conversion Ratio (Fcr) Of Japanese Quail Under Different Treatment Groups

Groups Weeks	T <sub>0</sub> (Control)	T <sub>1</sub> (MOLM-0.25%)	T <sub>2</sub> (MOLM-0.50%)	T <sub>3</sub> (MOLM-0.75%)	T <sub>4</sub> (MOLM-1.00%)
1 <sup>st</sup>	2.00	1.92	1.71	1.85	1.73
2 <sup>nd</sup>	2.07	2.03	2.14	1.97	2.33
3 <sup>rd</sup>	3.59	2.56	2.95	2.75	2.74
4 <sup>th</sup>	2.81	3.52	3.74	4.06	3.59
5 <sup>th</sup>	5.31	4.14	4.42	4.63	4.58
6 <sup>th</sup>	6.67	5.92	5.45	5.93	5.39
	3.64	3.35	3.39	3.53	3.42

The livability (Table 7.0) of all the groups is recorded as 98.33, 100, 98.33, 98.33 and 100% for T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively. The mortality percentage observed in this investigation can be considered as normal. These findings are in accordance with Safa and Tazi (2014) [31] who reported non-significant ( $P>0.05$ ) difference in

livability of broilers supplemented with *Moringa oleifera* leaf meal in the diet. The percent values of livability indicate that *Moringa oleifera* leaf meal have no detrimental effect on the health of Japanese quail.

**Table 7:** Per Cent Livability of Japanese quail Under Different Treatment Groups

Groups Parameter	T <sub>0</sub> (Control)	T <sub>1</sub> (MOLM-0.25%)	T <sub>2</sub> (MOLM-0.50%)	T <sub>3</sub> (MOLM-0.75%)	T <sub>4</sub> (MOLM-1.00%)
Livability (%)	98.33	100	98.33	98.33	100

The cost of production per Japanese quail was found to be Rs. 56.60, 55.83, 58.16, 58.02 and 55.60 for T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> groups, respectively (Table 8.0). Gross profit per Japanese quail was found to be highest in T<sub>1</sub> group (Rs. 12.13) followed by T<sub>2</sub> (Rs. 9.56), T<sub>4</sub> (Rs. 9.55), T<sub>3</sub> (Rs. 8.10) and T<sub>0</sub> (Rs. 4.12). Thus, among the experimental group the T<sub>1</sub> group showed best result in terms of

higher gross profit per Japanese quail. The higher gross profit per Japanese quail in T<sub>1</sub> might be due to better growth performance and improved FCR in T<sub>1</sub> group. These findings are in agreement with the findings of Dey and De. (2013)<sup>[9]</sup> where they have reported reduced production cost, higher body weight and improved FCR in *Moringa oleifera* treated groups as compared to control group.

**Table 8:** Costs of Production and Gross Profit (Rs) Per Quail under Different Treatment Groups

Experimental Groups	Items of expenditure			Total production cost (D) (A+B+C)	Production cost / bird (E)	Total saleable live weight (kg) (F)	II. Return (G)	III. Gross Profit (D - G) (H)	IV. Gross profit / bird (I)
	Chick cost (A)	Feed cost (B) Rs. 31.13/ Kg	Miscellaneous (C) 15% of (A+B)				1.Total saleable live weight x Rs.270 per kg(G)		
T <sub>0</sub> (control)	1360.00	1539.75	434.96	3339.71	56.60	13.27 (n=59)*	3582.90	243.19	4.12
T <sub>1</sub> (0.25% MOLM)	1360.00	1553.18	436.97	3350.15	55.83	15.06 (n=60)*	4066.20	716.05	12.13
T <sub>2</sub> (0.50% MOLM)	1360.00	1624.08	447.61	3431.69	58.16	14.80 (n=59)*	3996.00	564.31	9.56
T <sub>3</sub> (0.75% MOLM)	1360.00	1616.79	446.51	3423.30	58.02	14.45 (n=59)*	3901.50	478.20	8.10
T <sub>4</sub> (1.00% MOLM)	1360.00	1541.27	435.18	3336.45	55.60	14.48 (n=60)*	3909.60	573.15	9.55

\*n = Number of birds available for sale

Thus, it can be recommended that *Moringa oleifera* leaf meal can be used economically as a natural feed additive in Japanese quail diet at the level of 0.25 to 0.50% to improve its overall performance. The effective level of supplementation of *Moringa oleifera* leaf meal of 0.25 to 0.50% might be due to the hormesis effect. However, further in depth study involving more number of treatment birds and varying levels of *Moringa oleifera* leaf meal may be required to validate the present study.

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