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Influence of Moringa Oleifera Leaf Meal Used as Phytogenic Feed Additive on the Serum Metabolites and Egg Bioactive Compounds in Commercial Layers

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ABSTRACT

Phytogenic feed additives have been increasingly used in the last decade, and several plants and their metabolites have been investigated for the said purpose. In this context, present study aimed at evaluating the effects of *Moringa oleifera* as feed additive on layer performance, and egg bioactive compound levels and nutrient profile. HyLine W36 layers (n=200), 50 weeks of age, were randomly distributed in four treatments with five replicates of ten birds each. Four isocaloric (2725 kcal/kg) and isonitrogenous (CP 16%) diets were formulated and supplemented with 0, 0.5, 1.0, or 1.5% (w/w) of dried *Moringa oleifera* leaf powder (MLM). The results showed positive effects of MLM on egg production, egg mass, and feed conversion ratio, but negative effects on egg quality ($p \leq 0.05$). The contents of bioactive compounds, like β-carotene, quercetin, and selenium, in the diet and in the egg yolk were significantly ($p \leq 0.05$) higher in the group fed1.5% MLM, with values of 8.90, 48.88, and 0.54 mg/kg feed and 4906, 241 and 56.82 µg/100g yolk, respectively. Creatinine and glucose serum levels and cholesterol levels (serum and eggs) linearly increased as a function of increasing MLM dietary levels ($p \leq 0.05$). Antibody titers against Newcastle Disease significantly improved ($p \leq 0.05$) in the group fed the diet supplemented with 1.5% MLM. It was concluded that *Moringa oleifera* used as phytogenic feed additive enriches eggs with bioactive and functional compounds, and improves the production performance and the health status of layers.

INTRODUCTION

Additives are included in feeds to enhance animal performance and productivity, and for the prevention of different infections (Teteh *et al.,* 2013; Gould, 2008). Antibiotic growth promoters (AGP) have been used by the feed industry for decades, but have allegedly caused antibiotic resistance both in animals and humans beings, becoming a public health hazard (WHO, 2008). This was the basis for the ban on all types of AGPs in animal feeds in Europe and developed countries (Cogliani *et al.,* 2011), motivating the search for alternative growth promoters, such as phytogenic feed additives (Windisch *et al.,* 2008).

Phytogenic feed additives are plant-derived products that can modify the metabolism of healthy animals, ultimately affecting their growth and productivity. These additives also increase the levels of antioxidant and bioactive compounds in animal products (Windisch *et al.,* 2008). Bioactive secondary metabolites of plants, such as carotenoids, phenolic compounds, polyphenols, flavones, flavonoids, alkaloids, polypeptides, and essential oils have been shown to have anti-bacterial, antifungal, anti-aging, antioxidant, and functional properties (Cowan, 1999). In particular, it has been demonstrated that essential oils like cinnamaldehyde, eugenol, thymol, and carvacrol have antibacterial action against multiple pathogenic bacteria (Hernandez *et al.,* 2004; Tabak *et al.,* 1999). Such agents have been used for decades for the treatment and prophylaxis

of different diseases in humans and animals (Wallace *et al.,* 2010).

Moringa oleifera is rich in bioactive compounds, and may be a potential candidate as phytogenic feed additive (Joshi and Mehta, 2010). The synergistic combination of these compounds may positively and significantly influence the performance and productivity of livestock (Mbikay, 2012; Wallace *et al.,* 2010; Anwar *et al.,* 2007). Moringa leaves contain vitamins, flavonoids, and carotenoids, which not only serve as essential nutrients, but also enrich poultry meat and eggs, and intensify the pigmentation of the shanks and egg yolk (Melesse *et al.,* 2011; Fasuyi *et al.,* 2005). Considering the contents of bioactive compounds and essential nutrientsin *Moringa oleifera* leaves, they can be used both as a feed ingredient and asphytogenic feed additive to promote layer performance and to enrich the egg yolk with carotenoids, flavonoids, and selenium (Melesse *et al.,* 2011; Fasuyi *et al.,* 2005). These enriched eggs can be marketed as designer eggs or functional foods. Therefore, the objective of the present study was to analyze the effect of different levels of dried *Moringa oleifera* leaves powder on the production, immune response, and chemical composition of the egg yolk of commercial layers.

MATERIALS AND METHODS

Moringa leaf meal processing, birds, and experimental diets

Mature leaves of *Moringa oleifera* plants were collected, dried under a shade up to a moisture level of ≤ 12%, ground, and stored in polythene bags in a cool and dry place until further analysis and feed formulation (Banjo, 2012). The leaf meal was submitted to chemical analysis (macro and micro nutrients, bioactive compounds, and trace minerals) (Table 1).

Table 1 – Chemical composition of *Moringa oleifera* leaf meal

Chemical composition		Unit
Nutrients		
Moisture	7.60	g/100g
Crude Protein	26.93	g/100g
Ether Extract	6.84	g/100g
Ash	11.11	g/100g
Minerals		
Sodium	936	mg/100g
Potassium	2549	mg/100g
Calcium	2471	mg/100g
Magnesium	1041	mg/100g
Phosphorus	260	mg/100g
Selenium	2.88	mg/100g
Bioactive compounds		
Quercetin	312	mg/100g
β-carotene	56.23	mg/100g

Two hundred 50-week-old commercial layers having 66-65% egg production, were reared in battery cages on experimental farm of Faculty of Animal Production and Technology, University of Veterinary and Animal Science, Ravi Campus Pattoki, Punjab, Pakistan. Birds were randomly distributed into four treatments with five replicates (cage) of 10 birds each. The treatments consisted of diets supplemented with0, 0.5, 1.0 and 1.5% (w/w) of *Moringa oleifera* leaf meal (MLM) for a period of six weeks.

The four experimental diets (MLM-0%, MLM-0.5%, MLM-1.0%, and MLM-1.5%) were formulated to contain equal crude protein (16%) and of metabolizable energy (2725 kcal/kg) levels (Table 2). MLM was added on top of the basal feed over and above.

Table 2 – Ingredients and chemical composition of the experimental diets

Ingredients	Control	MLM 0.5 %	MLM 1.0 %	MLM 1.5 %
Maize	50.0	50.0	50.0	50.0
Soybean Meal 45%	23.75	23.75	23.75	23.75
Rice Polish (Fat >15%)	10.77	10.77	10.77	10.77
Limestone	10.03	10.03	10.03	10.03
Dicalcium phosphate	2.24	2.24	2.24	2.24
Soy Oil	2.0	2.0	2.0	2.0
L-Threonine	0.08	0.08	0.08	0.08
L-Lysine sulphate 55%	0.27	0.27	0.27	0.27
Salt	0.25	0.25	0.25	0.25
DL-Methionine	0.23	0.23	0.23	0.23
Sodium bicarbonate	0.18	0.18	0.18	0.18
Supplement1	0.2	0.2	0.2	0.2
Total	100	100	100	100
Moringa leaf meal (%)	0	0.5	1.0	1.5
Chemical composition	(%)	(%)	(%)	(%)
Dry Mater	90.26	90.26	90.26	90.26
Crude Protein	16.0	16.0	16.0	16.0
ME (kcal)	2725	2725	2725	2725
Fat	5.86	5.86	5.86	5.86
CF	3.85	3.85	3.85	3.85
Ash	11.74	11.74	11.74	11.74
Linoleic Acid	2.62	2.62	2.62	2.62
Dig. Lysine	0.85	0.85	0.85	0.85
Dig. Met + Cys	0.68	0.68	0.68	0.68
Dig. Threonine	0.57	0.57	0.57	0.57
Sodium	0.19	0.19	0.19	0.19
Total Phosphorus	0.81	0.81	0.81	0.81
Available Phosphorus	0.40	0.40	0.40	0.40
Calcium	4.50	4.50	4.50	4.50
Se (mg/kg)	0.14	0.27	0.41	0.54
β-Carotene (mg/kg)	0.31	3.12	5.84	8.90
Quercetin (mg/kg)	0.42	16.57	32.88	48.88

[1]Supplement (Per Kg of premix): vitamin A, 40,000 IU; Vitamin D3, 125,00 IU; Vitamin E, 250 IU; Vitamin C, 15.3 g; Vitamin K3 15 IU; Thiamine 10mg; Riboflavin 25 mg; Pyridoxine 20 mg; Pantothenic acid 60 mg; Folic acid 3.75 mg; Biotin 500 µg; Niacin 200 mg, Choline 2500 mg; Vitamin B12 60 µg; Co 0.2 mg; Cu 30 mg; Iodine 2.5 mg; Mn 300 mg; Mg 54 mg; Zn 30 mg; Fe 150 mg; Selenium 1.5mg; QS.

EVALUATED PARAMETERS

Feed intake and egg production

A feed allowance of 100 g/bird was daily supplied, and feed residues were daily recorded to determine weekly feed intake. Mortality was recorded on a daily basis. Feed conversion ratio and feed efficiency per dozen as well as per egg mass basis was calculated on a weekly basis.

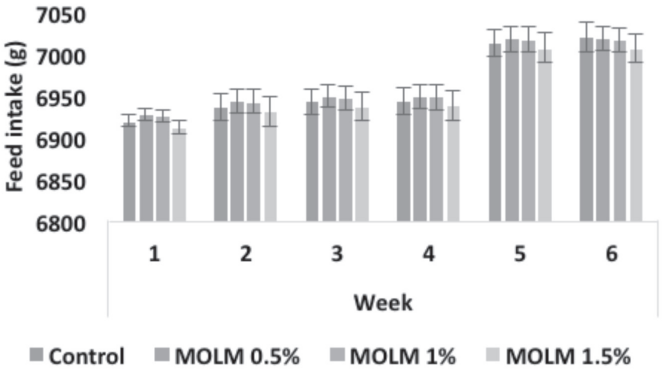

Figure 1 – Weekly feed intake of commercial layers fed different levels of Moringa oleifera leaf meal

Egg production was daily recorded, and egg mass, and egg weight were calculated on weekly basis. Egg quality traits were measured at the beginning of the experiment and every two weeks until the end of the experimental period in three eggs randomly collected per replicate, which was considered the experimental unit.

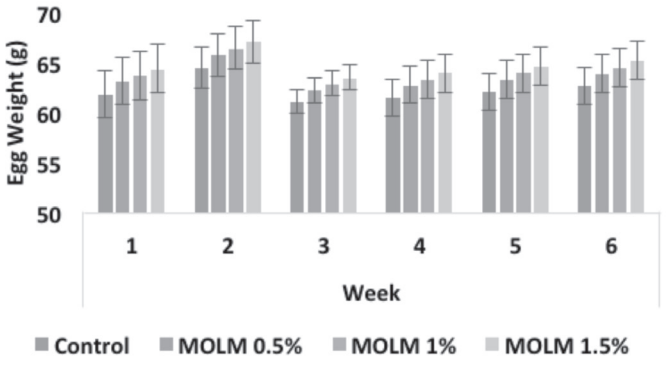

Figure 2 – Weekly egg weight of commercial layers fed different levels of Moringa oleifera leaf meal

Serum biochemical parameters and antibody titers

Blood samples were collected using sterile syringes containing anticoagulant from the three birds per replicate by wing web method on days 28 and 42 of the experiment. The blood samples were centrifuged, and the separated serum was stored until further analyses. Serum glutamic pyruvic transaminase (SGPT), alanine transaminase (ALT), and creatinine activities, and cholesterol level were measured using specific protocols of a commercial kit (Merck Microlab-300 in WTO Laboratory, UVAS, Lahore, Pakistan). Serum samples were also analyzed for antibody titers against Newcastle disease by hemagglutination (HA) and hemagglutinin-inhibition (HI) technique (Daniel & Seal, 1998).

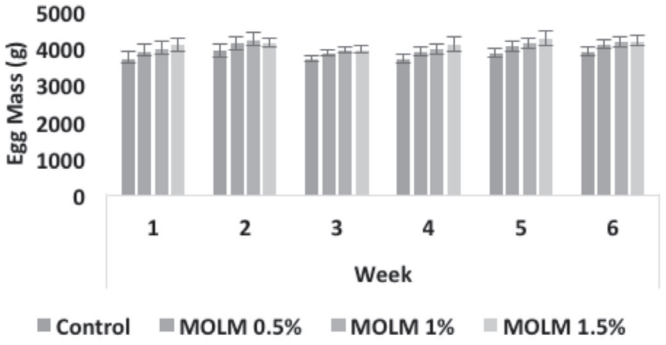

Figure 3 – Weekly egg mass of commercial layers fed different levels of Moringa oleifera leaf meal

Egg chemical composition

The chemical analysis of the egg yolk was performed to estimate moisture, crude protein, ash, ether extract, and fiber contents according to standard methods (AOAC, 2005). Egg yolks were submitted to wet digestion, as described by AOAC (2005) to determine Na, K, Ca, Mg, and Se contents.

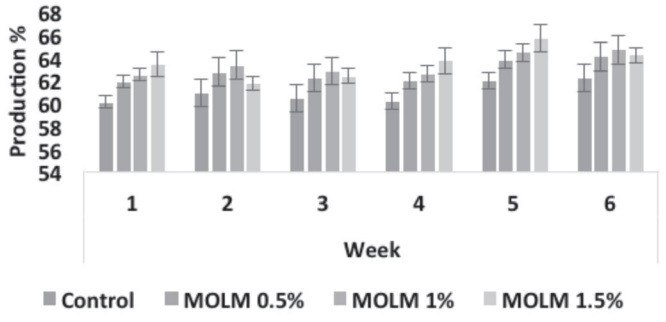

Figure 4 – Weekly egg production % of commercial layers fed different levels of Moringa oleifera leaf meal

Egg yolk β-carotene content

Egg yolk β-carotene content was determined by HPLC according to the method described by Saini *et al.* (2014) and Farida *et al.* (2008). Briefly, 1 g egg yolk was mixed with methanol (8mL) and 2 mL of 1N HCL. The sample was then vortexed for 5 min and the procedure was repeated thrice. The mixture was then centrifuged at 4000 rpm for 15 min, and the supernatant was removed and placed on water bath for drying. The residue was dissolved in 1 mL of the mobile phase solution

(acetonitrile:dichloromethane:methanol, 70:20:10, v/v/v). The samples were filtered (Whatman, No. 40,0.1.0 µm filter), eluted through a column (C_{18}, 5 µm, 250 mm × 4.6 mm) at a flow rate 1.0 mL/min, and detected at 450 nm using diode array detector (DAD). Beta carotene contents were determined from a calibration curve of a range of standard solutions.

Egg yolk quercetin content

Yolk quercetin content was determined according to the method of Tokusoglu *et al.* (2003), with a slight modification. Briefly, 1 g egg yolk was taken and mixed with acidified methanol. The temperature of the sample was lowered, and the extract was centrifuged at 1500g and 5000rpm. The supernatant was removed and sonicated, and finally placed in HPLC vials. A sample volume of 20µL was injected, and elution was carried out through aC_{18} column (250 × 4.6 mm; 5 µm particle size). The mobile-phase solution consisted of two solvents in equal proportion; A (3% tri-fluoro acetic acid) and B (80:20 v/v of acetonitrile and methanol). The flow rate was kept at 1.0 mL/min.

Egg yolk cholesterol content

Egg yolk cholesterol content was determined according to the method described by the AOAC (2005). Briefly, a solution of acetone and egg yolk (1: 1 ratio) was vigorously shaken for 2 min, centrifuged thrice, and the supernatant were evaporated. Cholesterol was de-esterified using cholesterol esterase. For this purpose, the acetone fraction was dissolved in a few mL (known value) of isopropanol. Then, 1 mL of the sample was placed in another test tube, 5mL of isopropanol was added, and the solution was vortexed. The sample was then mixed with cholesterol reagent, and its absorbance was measured at 500 nm after 10 min. Cholesterol was quantified using a calibration curve.

Statistical analysis

Data were analyzed using one-way analysis of variance (PROC GLM in SAS software, SAS Inc. 9.4), and means were compared by Duncan's Multiple Range test. P-values lower than 0.05 were considered significantly different.

RESULTS AND DISCUSSION

Live performance

Dietary MLM levels positively ($p \leq 0.05$) the performance of commercial layers. Egg mass and production percentage linearly increased as dietary MLM supplementation levels increased (Table 3). Statistically, egg mass and egg production were not different the hens fed the MLM-supplemented diets, but tended to increase as MLM levels increased. Feed conversion ratio per kg egg mass and per dozen eggs linearly decreased as dietary MLM levels increased (Table 3). The best FCR values, both per dozen and per egg mass, were obtained with the 1.5% MLM diet (Fig. 5&6). Total feed intake, egg weight, and livability were not affected by the treatments (Table 3).

Table 3 – Production performance and egg characteristics of commercial layers fed on different levels of *Moringa oleifera* leaf meal for 6 weeks (55-61 weeks)

Parameter	Control	MLM 0.5 %	MLM 1.0 %	MLM 1.5 %
Feed Intake (g)	41.77±0.04	41.80±0.04	41.79±0.04	41.72±0.05
Egg Mass (Kg)	22.74±0.35^{b}	23.88±0.37^{ab}	24.35±0.37^{a}	24.65±0.48^{a}
Egg weight (g)	62.22±0.88	63.47±0.90	64.09±0.91	64.73±0.92
Egg production %	60.88±0.70^{b}	62.71±0.72^{ab}	63.32±0.72^{a}	63.43±0.47^{a}
FCR/dz eggs	1.37±0.02^{a}	1.33±0.01^{ab}	1.32±0.01^{b}	1.32±0.01^{b}
FCR/egg mass	1.85±0.03^{a}	1.76±0.03^{b}	1.72±0.03^{b}	1.70±0.03^{b}
Egg Characteristics				
Shape Index	79.37±0.37^{a}	78.37±0.48^{ab}	77.95±0.52^{b}	79.42±0.39^{a}
Surface Area (cm^2)	71.63±1.33	72.66±1.24	70.49±1.46	69.86±1.55
Volume (cm^3)	51.52±1.41	52.61±1.34	50.32±1.57	49.67±1.62
Yolk Index	38.38±0.84^{a}	34.93±0.51^{b}	34.56±0.88^{b}	35.02±0.51^{b}
Haugh Unit Score	106.68±0.57^{a}	103.99±0.52^{b}	103.01±0.72^{b}	102.87±0.34^{b}
Shell Thickness (mm)	0.36±0.01^{ab}	0.37±0.01^{a}	0.35±0.01^{ab}	0.34±0.01^{b}

Note: Superscripts indicate significant differences among means in the same row (P≤0.05)

The slight increasing egg mass and egg production trends may be attributed to extra amino acids, such lysine and methionine, supplied by MLM (Fakhraei *et al.,* 2010). As previously reported, egg production improved when the diet was supplemented with additional 0.50 to 0.64% lysine (Alebachew *et al.,* 2016). Due to its bioactive compounds, including antioxidants, phytoestrogens and essential amino

acids, MLM positively influences egg mass, FCR and egg production (Liu *et al.,* 2014; Mohammed *et al.,* 2012). On the other hand, studies reported that MLM supplementation did not affect egg production or quality (El-Sheikh *et al.,* 2015; Abou-Elezz *et al.,* 2011), which may be attributed to the poor digestibility of higher MLM dietary levels due presence of fiber and some anti-nutritional factors.

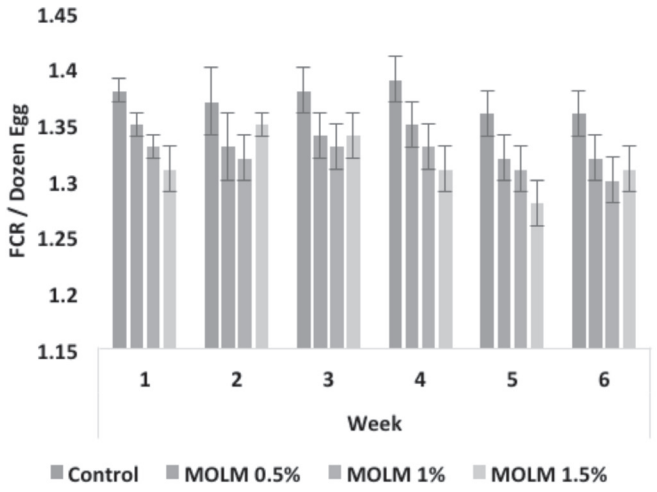

Figure 5 – Weekly FCR/dozen eggs of commercial layers fed different levels of Moringa oleifera leaf meal

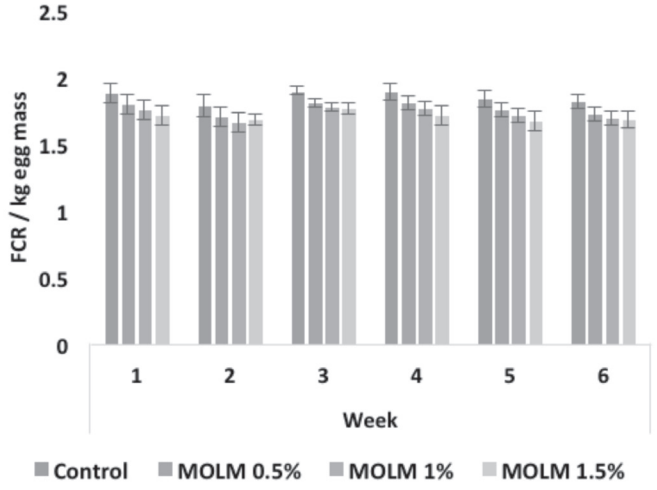

Figure 6 – Weekly FCR/kg egg mass of commercial layers fed different levels of Moringa oleifera leaf meal

Egg quality

The results of the present study showed that egg yolk index, Haugh unit and eggshell thickness linearly decreased as MLM levels increased, with the lowest values recorded in the egg of hens fed the highest MLM level (Table 3). Egg shape index showed a quadratic response, and was the highest in the 1.5% MLM-supplemented group (Table 3). During the experimental period, egg surface area and volume remained unchanged (Table 3).

Limitations in the use of plant-based feed additives or ingredients are due to anti-nutritional factors, as in *Moringa oleifera* there is high content of fiber, saponins, phytoestrogens and many other compounds (Makkar & Becker, 1997). When the dose rate is increased these compounds hinder normal metabolism and affect the production, shell thickness and overall egg (El-Sheikh *et al.,* 2015; Abou-Elezz *et al.,* 2011). Some other studies report that antioxidants positively affect egg production (Liu *et al.,* 2014; Mohammed *et al.,* 2012).

Bioactive compounds in the egg yolk

β-*carotene and quercetin*

Egg yolk β-carotene and quercetin levels linearly increased with MLM dietary supplementation (Table 4). The highest ($p \leq 0.05$) β-carotene values (4,906 mg/100g yolk and 8.90 mg/kg feed) and quercitin values (241 µg/100g yolk and 48.88 mg/kg feed) were obtained in the eggs of the hens fed the highest MLM supplementation level (1.5% MLM).

MLM is rich in carotenoids and flavonoids, which are very strong natural antioxidants. The observed enrichment of the egg yolk with β-carotene and quercetin may be attributed to the high content of these compounds (15.25 mg of β-carotene and 100 mg of quercitin in 100 g dried leaf) in *Moringa oleifera* leaf meal (Tesfaye *et al.,* 2014; Lako *et al.,* 2007). Most of the β-carotene is deposited in the egg yolk, whereas quercetin is also deposited in the egg albumen chelated with amino acids. The similar results were reported in other studies evaluating the use of canthacol, MLM, tomato peel, colored carrots ,and apple skin for the enrichment of egg yolks with β-carotene and quercetin (Gakuya *et al.,* 2014; Liu *et al.,* 2014; Olson *et al.,* 2008).

Selenium

The results of the present study showed that egg yolk selenium content linearly increased with increasing dietary MLM supplementation levels MLM ($p \leq 0.05$). The egg yolks of the hens fed the control diet, not supplemented with MLM, contained the lowest selenium values, whereas the highest values were recorded with 1.5% MLM diet (Table 4). Yolk selenium increased up to 56.82 µg/100g of egg yolk when the feed offered contained 0.54mg/kg of organic selenium. This higher level of selenium enrichment was obtained by feeding selenium as of selenomethionine and selenocystine, which have better bioavailability and tissue retention (Delezie *et al.,* 2014). *Moringa oleifera* leaves and pods contain 2.88mg and 25.7 mg/100g of Se per 100g of dried leaves, respectively

(Table 4). Many researchers have used selenium yeast, selenomethionine and sodium selenite for egg yolk enrichment (Delezie *et al.,* 2014; Wang *et al.,* 2010).

Cholesterol

MLM supplementation in layer feeds had a significant and positive impact on the egg lipid profile ($p \leq 0.05$). Total cholesterol in the yolk linearly decreased with the MLM supplementation level, and was the lowest when hens were fed 1.5% MLM (Table 4). Plants are enriched with phytosterols, which decrease both egg and serum cholesterol levels (Liu *et al.*, 2010). This reduction in cholesterol levels is attributed to plant sterols. *Moringa oleifera* is enriched with β-sitosterol, which is responsible for this activity (Hussain *et al.,* 2014). Egg cholesterol level is also influenced by antioxidants (flavonoids and carotenoid) in the diet (Benakmoum *et al.,* 2013).

Table 4 – Levels of bioactive compounds and selenium in the egg yolk and in the experimental diets.

Parameter	Control	MLM 0.5 %	MLM 1.0 %	MLM 1.5 %
Diet Sample				
β-carotene (mg/kg)	0.31±0.01^{d}	3.12±0.03^{c}	5.84±0.02^{b}	8.90±0.03^{a}
Quercetin (mg/kg)	0.42±0.02^{d}	16.57±0.17^{c}	32.88±0.09^{b}	48.88±0.20^{a}
Selenium (mg/kg)	0.14±0.00^{d}	0.27±0.00^{c}	0.41±0.00^{b}	0.54±0.00^{a}
Yolk Sample				
β-carotene (µg/100g)	293.2±7.11^{d}	2964.6±27.08^{c}	4716.3±14.84^{b}	4906.4±14.37^{a}
Quercetin (µg/100g)	2.07±0.11^{d}	81.67±0.83^{c}	162.11±0.45^{b}	241.00±1.00^{a}
Selenium (µg/100g)	14.27±0.19^{d}	28.47±0.19^{c}	42.67±0.19^{b}	56.82±0.19^{a}
Cholesterol (µg/100g)	223.54±0.74^{a}	221.30±0.73^{b}	216.83±0.72^{c}	205.99±0.68^{d}

Note: Superscripts indicate significant differences among means in the same row ($p \leq 0.05$)

Egg yolk chemical analysis and mineral profile

The nutrient profile of the egg yolk was significantly affected with dietary MLM supplementation ($p \leq 0.05$). Moisture and ether extract levels linearly decreased as supplementation levels increased, with the lowest levels recorded for the MLM-1.5% group (Table 5). Protein and ash contents linearly increased with MLM supplementation rate, with the highest values recorded for the group fed the MLM-1.5% diet (Table 5). The levels of antioxidants, flavonoids, carotenoids, lysine and methionine, as well as protein and energy of MLM may be responsible for the above response (Nkukwana *et al.,* 2015).

Egg yolk mineral profile was significantly affected by MLM supplementation levels in the diets ($p \leq 0.05$). Sodium level was lowest in the control and highest in the groups supplemented with MLM at 1.0% and 1.5% (Table 5). In addition of sodium, the yolk levels of other minerals like potassium, calcium, magnesium, and phosphorus linearly increased as MLM supplementation levels increased, with the lowest levels of these minerals recorded in the egg yolk of layers fed the basal diet (Table 5). These results may be attributed to MLM ash content, as shown in previous studies (Nkukwana *et al.,* 2015; Qwele *et al.,* 2013).

Table 5 – Nutrient and mineral profile of egg yolks of commercial layers fed different levels of Moringa oleifera leaf meal.

Parameter	Control	MLM 0.5 %	MLM 1.0 %	MLM 1.5 %
Nutrients[1]				
Moisture	49.55±0.10^{a}	48.72±0.10^{b}	47.44±0.06^{c}	47.14±0.11^{d}
Crude Protein	16.54±0.08^{c}	17.37±0.08^{b}	17.89±0.09^{a}	17.91±0.08^{a}
Ash	1.45±0.02^{b}	1.49±0.02^{ab}	1.51±0.02^{a}	1.51±0.02^{a}
Ether Extract	32.99±0.15^{a}	32.01±0.15^{b}	31.05±0.14^{c}	31.05±0.14^{c}
Mineral Profile[2]				
Sodium	61.31±0.62^{ab}	62.62±0.60^{a}	60.75±0.58^{b}	60.75±0.58^{b}
Potassium	111.17±0.35^{c}	114.51±0.36^{b}	117.95±0.38^{a}	117.95±0.38^{a}
Calcium	136.36±0.31^{c}	140.45±0.32^{b}	143.26±0.33^{a}	143.26±0.33^{a}
Magnesium	12.61±0.05^{c}	13.03±0.05^{b}	13.37±0.03^{a}	13.30±0.03^{a}
Phosphorus	396.03±4.17^{b}	407.92±4.30^{ab}	411.99±4.34^{a}	411.99±4.34^{a}

Note: Superscripts indicate significant differences among means in the same row ($p \leq 0.05$); [1]Expressed in g/100g, [2]Expressed in mg/100g

Serum biochemistry and immune response

The dietary supplementation of MLM significantly influenced serum biochemical parameters ($p \leq 0.05$). The lowest SGPT, creatinine and cholesterol values were observed in the hens fed the highest MLM level (1.5%), whereas the lowest values were recorded in the control group (Table 6). Serum glucose levels linearly decreased with increasing MLM dietary levels, and were the lowest in the 1.5% MLM group (Table 6). Flavonoids improve liver and kidney function, improving nutrient digestion. *Moringa oleifera* is rich in flavonoids and carotenoids (β-carotene) which positively affect SGPT, creatinine and glucose levels in the serum (Elkloub *et al.,* 2015). Phytosterols (β-sitosterol) of *Moringa oleifera* reduced serum cholesterol levels of rats (Ghasi *et al.,* 2000).

Newcastle disease titers were significantly influenced by dietary MLM levels, and the highest values were observed in the group fed the MLM-1.5% diet, whereas the lowest titers were recorded in the control group ($p \leq 0.05$) (Table 6). Antioxidants play a key role in the immune function, as they affect the gut environment by inhibiting the growth of pathogenic microbes as well as the production of endotoxins. The higher antibody titers obtained may be attributed to the effects of antioxidants and essential amino acids and to the higher levels of organic trace minerals (Elkloub *et al.,* 2015; Saei *et al.,* 2013; Yang *et al.,* 2006).

Table 6 – Serum chemistry and antibody titers of commercial layer fed with different levels of Moringa oleifera leaf meal

Parameter	Control	MLM 0.5 %	MLM 1.0 %	MLM 1.5 %
Blood metabolites (week 4)				
SGPT	25.21±0.37^{a}	19.37±0.67^{b}	13.91±0.35^{d}	15.81±0.42^{c}
Glucose	268.27±1.56^{a}	250.47±0.83^{b}	239.67±0.73^{c}	248.40±1.13^{b}
Creatinine	1.64±0.01^{a}	1.27±0.03^{b}	1.09±0.01^{d}	1.19±0.01^{c}
Cholesterol	160.07±1.36^{a}	137.60±1.17^{b}	85.00±1.41^{c}	81.47±1.13^{c}
Blood metabolites and antibody titers (week 6)				
SGPT	24.71±0.36^{a}	18.98±0.65^{b}	13.63±0.34^{d}	15.49±0.41^{c}
Glucose	262.90±1.53^{a}	245.46±0.82^{b}	234.87±0.72^{c}	243.43±1.11^{b}
Creatinine	1.61±0.01^{a}	1.24±0.03^{b}	1.07±0.01^{d}	1.17±0.01^{c}
Cholesterol	156.87±1.33^{a}	134.85±1.14^{b}	83.30±1.38^{c}	79.84±1.11^{c}
NDV titers	32.00±4.68^{b}	38.40±3.42^{ab}	38.40±5.80^{ab}	51.20±4.19^{a}

Note: Superscripts indicate significant differences among means in the same row ($p \leq 0.05$); SGPT: U/L; glucose, creatinine, and cholesterol: mg/dL

CONCLUSIONS

The results of the present study showed that *Moringa oleifera* used as a phytogenic feed additive enriches eggs with bioactive and functional compounds, and improves the production performance and the health status of layers. Moreover, it may add value to the eggs by reducing their cholesterol levels and enhancing egg shelf life.

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CONFLICT OF INTEREST

No potential conflict of interest was found by the authors.

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