CSIRO PUBLISHING

Animal Production Science
https://doi.org/10.1071/AN19671

Performance of broilers and layers supplemented with *Moringa stenopetala* leaf meal under hot humid tropical conditions

Metekia Tamiru[A,C], *Shiferaw Ashagrie*[A], *Ashraf Alkhtib*[B,C,D], *Minyehun Getachew*[A], *Solomon Demeke*[A], *Waseyehun Hassen*[A], *Zemene Worku*[A] *and Emily Burton*[B]

[A]Department of Animal Science, College of Agriculture and Veterinary Medicine, Jimma University, BO Box 378, Jimma, MRPJ+2C Jimma, Ethiopia.
[B]Nottingham Trent University, School of Animal, Rural and Environmental Sciences, Brackenhurst Campus, Southwell, Nottinghamshire, NG25 0QF, UK.
[C]Both authors contributed equally to this paper.
[D]Corresponding author. Email: a.s.alkhtib@gmail.com

Abstract

Context. Heat stress poses a major limit to poultry production. *Moringa stenopetala* leaf meal (MSL) could be a promising feed additive for poultry raised under heat stress, as it is rich in antioxidants.

Aims. To determine the effect of (MSL) supplementation on the production and quality of broilers under heat-stress conditions.

Methods. In the broiler performance trial, 156 1-day old Cobb 500 broilers were assigned to four groups, with each containing three replicates of 13 chicks each. For the egg production trial, 108 Bovan Brown layers aged 20 weeks were allocated to four groups, with each containing three replicates of nine hens. The four groups of broiler and layer chicken were supplemented with MSL at a level of 0% (control), 1% (MSL1), 1.5% (MSL1.5) and 2% (MSL2). Relative humidity, and minimum and maximum temperatures were 82%, 13°C and 23°C respectively. The effects of MSL supplementation on growth performance, characteristics of digestive organs, carcass traits, egg production and egg quality were analysed with one-way ANOVA.

Key results. *Moringa stenopetala*-leaf supplementation at a level up to 2% (MSL2) did not significantly affect feed intake, weight gain or feed conversion ratio of the birds. Supplemented broilers had a significantly longer large intestine (24% for MSL1, 37% for MSL1.5 and 49% for MSL2) and a heavier pancreas (82% for MSL1, 67% for MSL1.5, and 57% for MSL2) than did the control broilers. Hot and cold carcass weights, dressing yield, dressing percentage, breast-meat weight, drumstick and thigh-meat weights, wing-meat weight, back weight and meat pH were not significantly affected by MSL supplementation. Ash content of meat of the MSL2-fed birds was significantly higher than that of the control birds (3.51% vs 2.74% respectively). Egg production, feed conversion ratio and interior and exterior egg-quality parameters were not significantly affected by MSL supplementation. Intensity of yolk colour was significantly and linearly enhanced due to MSL supplementation (by ~5–8 times compared with the control).

Conclusions. Supplementation with MSL at a level up to 2% improved yolk colour of Bovan Brown layer eggs, with no effect on meat and egg production.

Implications. Yolk colour of eggs of layers raised under heat stress can be improved by MSL supplementation at 1%, with there being a minimum increase in the diet cost.

Additional keywords: Bovan Brown layers, Cobb 500, heat, humidity, stress, tropics.

Received 25 November 2019, accepted 5 June 2020, published online dd mmm yyyy

Introduction

Extensive chicken rearing systems contribute significantly to food security of rural livelihoods in most developing countries (Tadelle *et al*. 2000). Thus, improving poultry production would play a key role in upgrading the overall livelihoods of poor farmers in developing countries (Simainga *et al*. 2011).

Heat stress in chickens caused by hot and humid conditions hampering the birds in dissipating the heat produced through metabolism (Webster 1983) is a major limiting factor to poultry production in the tropics. Heat stress in chickens could be decreased by increasing ventilation and the use of evaporative cooling systems; however, such solutions are

costly to farmers in the developing countries. Nutritional modification may present a sustainable and cost-effective alternative strategy.

Moringa stenopetala is indigenous to eastern Africa, Central America and Southeast Asia and is cultivated for its leaf, which has a high biomass yield (~21 t DM/ha; Mendieta-Araica *et al*. 2013) and nutritive and medicinal value (Melesse *et al*. 2013). *Moringa stenopetala* leaf (MSL) has a high content of crude protein (CP), minerals, vitamin and essential amino acids (Melesse *et al*. 2012) and a low content of cell-wall constituents. Additionally, a low concentration of tannins (4.1%) and oxalates (3.1%) was reported in *Moringa* leaf (Heuzé *et al*. 2015). *Moringa* leaf has been introduced successfully to broiler diets, but its efficacy seems highly variable across several parameters. It has been reported that supplementing the diet of broilers with *M. oleifera* leaf powder at a supplementation level of 1.2% enhances gut health without a significant improvement in growth (Khan *et al*. 2017). Broilers fed on a diet containing 1.5% *M. oleifera* leaf showed an improvement in growth performance and meat quality (Ahmad *et al*. 2017). Cui *et al*. (2018) reported that *M. oleifera* leaf meal could be integrated into broiler diet at a level of 1.56% to improve fatty acid profile and colour of meat, without there being any penalty on growth performance. Inclusion of *M. oleifera* leaf meal in the broiler diet at a level of 0.1% enhances humoral immune response (Rama Rao *et al*. 2019). It has been shown that the use of *M. oleifera* leaf meal at a level of 2.5% of broiler diet does not alter nutrient digestibility, but improves body growth (Nkukwana *et al*. 2014). Using MSL in total replacement of roasted soybeans at a level of 14% in the diet of Koekoek chickens has been shown to leverage growth performance and carcass yield (Melesse *et al*. 2013). Adding *M. oleifera* leaf at a concentration of 0.5% has been shown to improve weight gain, feed conversion ratio, dressing percentage and meat quality of broilers (Karthivashan *et al*. 2015). Many studies have reported on the positive effect of *M. oleifera* leaf on laying performance (Abouelezz *et al*. 2011; Swain *et al*. 2017). Inclusion of *M. oleifera* leaf at 5% of the layer diet in replacement of soybean meal has been shown to significantly improve laying performance and egg quality (Alebachew *et al*. 2016). Supplementing hens by 1% *M. oleifera* leaf has been shown to improve laying rate and egg mass (Teteh *et al*. 2016). However, layers fed on 75 g DM of *M. oleifera* leaf did not have better laying performance and egg quality than did the control group in the study of Abou-Elezz Fouad Mohammed *et al*. (2012). Inclusion of *M. oleifera* leaf at a level of 5% was shown to improve yolk colour, but it did not enhance laying performance and egg quality in the study of Lu *et al*. (2016). As *Moringa* leaf is rich in vitamins, essential amino acids and antioxidants, supplementing broilers and layers with MLS under heat-stress conditions is expected to improve production performance.

To our knowledge, no studies have evaluated the use of MSL to improve production performance and quality of meat and egg of chickens in temperate climates. Accordingly, the goal of the present study was to identify the effect of MSL supplementation on growth performance and carcass traits of Cobb 500 and on egg production and quality of Bovan Brown hens under heat-stress conditions.

Materials and methods

Study site

The experiment was conducted at Jimma University College of Agriculture and Veterinary Medicine poultry farm, Ethiopia (7°40′N, 36°50′E, 1750 masl), in August 2018. The average humidity during the study was 82% and the mean minimum and maximum temperatures were 13°C and 23°C respectively.

Collection and preparation of M. stenopetala leaf

Branches and twigs were collected from 6–7-year-old *M. stenopetala* trees in Konso area, southern Ethiopia (1400–2300 masl, annual rainfall 300–900 mm, average minimum and maximum temperatures 15°C and 33°C respectively; Ferro-Vázquez *et al*. 2017); then, the leaves were handpicked, sundried for 5 days directly under sun rays and ground using a mortar mill with a sieve size of 4 mm and, finally, stored until used.

Experimental diets and experimental design

In total, 156 unsexed 1-day-old chicks of commercial Cobb 500 broilers were used in the study. The chicks were weighed and randomly grouped into four groups of 39 chicks each. Each group was then subdivided into three groups (replicates) of 13 chicks. Group 1 (control), Group 2 (MSL1), Group 3 (MSL1.5) and Group 4 (MSL2) were supplemented with 0%, 1%, 1.5% and 2% of MSL respectively (added on the top of the diet). Standard starter and finisher commercial concentrates (mashed), formulated to meet nutrient requirements of Cobb 500 as per recommended by NRC (1994), were purchased from Alema Poultry PLC, Debre Zeit, Ethiopia, and used in the current study. Chemical analysis and metabolisable energy of the experimental feeds are presented in Table 1.

In total, 108 18-week-old Bovan Brown hens were randomly allotted to four treatments (as per in broiler trial) of three replicates and nine birds per replicate. Birds in each replicate were randomly allocated to three cages. The basal diet was a concentrate formulated to meet nutrient requirements of Bovan Brown layers (NRC 1994). The trial lasted for 56 days, in addition to 7 days of adaptation.

The birds of the study and the commercial feed were bought from Alema poultry PLC.

Table 1. Chemical composition and metabolisable energy content of the experimental feeds

CF, crude fibre (%DM); CP, crude protein (% DM); EE, ether extract (%DM); ME, metabolisable energy (MJ/kg DM) calculated according to Wiseman (1987); and NFE, nitrogen-free extract (% DM)

Parameter	DM	CP	EE	Ash	CF	NFE	ME
Moringa stenopetala	90.6	29.3	5.15	12.5	11.5	32.2	11.8
Broiler diet							
Starter (0–21 days)	90.5	22.4	8.97	4.12	9.45	45.6	12.5
Finisher (22–42 days)	93.6	20.5	8.76	4.32	9.23	50.8	12.7
Layer diet	90.9	16	6.62	8.56	6.64	62.8	14.1

Management of the experimental birds

The study was approved by the Ethical Committee of Jimma University, Ethiopia. All birds were vaccinated against Marek's disease, Newcastle disease, infectious bursal disease (Gumboro), fowl typhoid and fowl pox, as per the recommended vaccination schedules of the hybrid. Chicks were raised in a deep-litter housing system, with concrete floors covered by wood shavings at a depth of 5 cm. Watering and feeding troughs of every pen were cleaned and disinfected using appropriate detergents and insecticides. The birds were fed twice a day *ad libitum*. The daily intake of the birds was calculated by subtracting the leftover feed from the offered feed. Clean water was provided *ad libitum* throughout the experimental period and appropriate ventilation was maintained in all pens. The lighting regimen in the room started as 23 h light on Day 1, with darkness increasing by 1 h/day until 6 h of darkness was reached, then maintained throughout the rest of the study. All necessary biosecurity measures were put in place.

Bird tissue and feed analysis

Representative sample of the feedstuffs and chicken meat materials were ground to pass through 1-mm screen then analysed for DM, ash, CP, ether extract (EE) and crude fibre (CF), according to AOAC (2006). Total nitrogen content of the feed was determined using micro-Kjeldahl method and, then, CP was calculated as nitrogen × 6.25. Nitrogen free extract was calculated by subtracting EE, CF and CP from organic matter. Metabolisable energy of the experimental feeds was calculated according to Wiseman (1987). All samples were analysed at Jimma University, Animal Nutrition and Post-harvest and Food Science Technology Laboratory.

At the end of the egg-production trial, blood samples were collected from the jugular vein of three hens per replicate into heparinised tubes, centrifuged (spectrafuge centrifuge, 1677*g* for 20 min at 4°C) and the plasma was stored at –15°C. Concentrations of high-density lipoprotein, low-density lipoprotein and triglyceride were determined at Universal Clinic Chemical Pathology Diagnostic Laboratory, Jimma, Ethiopia, following procedures of Baron and Warren (1997).

Data collection

Growth performance and carcass traits

The daily intake by the birds was calculated by subtracting the leftover feed from the offered feed. The bodyweight of birds was recorded at the start of the experiment and then on a weekly basis at 0700 hours before the morning feed; then, bodyweight gain and feed conversion ratio were calculated.

At the end of the growth trial, two birds from each replicate were randomly selected and kept in separate temporary pens, leg tagged, starved overnight, then weighed (slaughter weight) and killed on the next day by severing the jugular vein to allow complete bleeding. The birds were manually defeathered, eviscerated and cut up according to standard procedures. Dressing percentage was recorded as the percentage of hot carcass weight from slaughter weight. Measurements of individual digestive organs and weights of carcass cuts were also recorded.

Laying performance and egg quality

The initial and the final liveweights of hens were recorded. Egg production, egg weight and feed consumption of every replicate were recorded daily to calculate egg mass, egg production, rate of lay, feed intake and feed conversion ratio.

Three eggs were randomly collected from each replicate on a weekly basis to assess egg interior and exterior qualities. Shell weight, shell strength (measured by an egg force reader (EFR-01; Orka Food Technology, West Bountiful, UT, USA)), shell thickness (determined by eggshell thickness gauge (Robotmation Co.Köyliö, Finland) at the large end, equatorial region, and small end) and specific gravity were determined.

A Roche colour fan (Hoffman-La Roche, Basel, Switzerland) was used to determine the egg-yolk colour. Height of albumin and yolk (measured with an electronic digital caliper (SH14100025; Shenhan, Shanghai, China), albumin weight and yolk weight were measured. Haugh unit was calculated according to Card and Nesheim (1972).

Antioxidant analyses of plasma, meat and egg

Two birds per replicate were randomly selected and blood samples were collected from the wing vein, centrifuged at 3000*g* for 10 min at 4°C then stored at –20°C. Plasma was analysed for antioxidative capacity (AOC), superoxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde according to (Wang *et al*. 2015), by using reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Samples of breast and thigh meat were homogenised with four-fold saline (0.9% NaCl solution stored at 4°C overnight), centrifuged (10 min at 2800*g* at 4°C), and the supernatant was collected.

Muscle protein was measured with the biuret method by using the instructions of the total protein reagent (Nanjing Jiancheng Bioengineering Institute). The malondialdehyde concentration of meat samples was assayed by chemical colorimetric diagnostic kits (Nanjing Jiancheng Bioengineering Institute) and expressed as nmol of malondialdehyde per milligram protein (Zhang *et al*. 2010).

For determination of egg-yolk oxidative stability, three eggs from each cage were stored at room temperature for 15, 20 and 30 days. Eggs were broken at the end of the storage periods and their yolks were separated and dry frozen. Yolk oxidation was analysed by the thiobarbituric acid-reactive species method according to Vyncke (1970), with the modifications of Ramanathan and Das (1992).

Statistical analyses

The effect of MSL supplementation on growth performance, skin colour, carcass characters, and egg production and quality was analysed using one-way ANOVA. Means of treatments were compared using least significant-difference test at $P \leq 0.05$. All statistical analyses were performed using Statistical Analysis System (SAS 2012).

Results

Growth performance

Growth performance of broilers in the control group as well as in the experimental treatments is presented in Table 2. There was no significant increase in feed intake and no improvement in bodyweight gain or feed conversion ratio were found as a result of an increased level of MSL supplementation. A small but no significant improvement in bodyweight gain and feed conversion ratio was found. Table 3 shows that feed intake, bodyweight gain, and feed conversion ratio during finishing were not significantly altered by MSL supplementation.

Digestive organs

Measurements of digestive organs of the broilers are presented in Table 4. Length of large intestine, weight of pancreas and weight of jejunum were significantly affected by MSL supplementation, while the measures of other organs were not.

Large intestine of birds in MSL1, MSL1.5 and MSL2 was 1.49%, 1.36% and 1.24% longer respectively, than that of the

Table 2. Growth performance of Cobb 500 broilers supplemented with *Moringa stenopetala*

Control, 0% of moringa leaf meal; MSL1, control + 1% moringa leaf meal; MSL1.5, control + 1.5% moringa leaf meal; MSL2, control + 2% moringa leaf meal. s.e.m., standard error of the mean

Time	Treatment				
	Control	MSL1	MSL1.5	MSL2	s.e.m.
			Feed intake (g)		
1st week	131	136	137	138	5.07
2nd week	362	361	366	369	14.1
3rd week	576	576	578	578	26
4th week	790	791	794	798	32.4
5th week	929	951	950	965	44.2
6th week	968	973	979	984	43.7
			Bird weight (g)		
0 days	43.6	43.6	43.5	43.7	2.59
1st week	165	166	166	166	6.93
2nd week	408	409	417	416	15.9
3rd week	804	807	827	814	45.1
4th week	1270	1275	1334	1310	86.5
5th week	1723	1784	1851	1818	125
6th week	2215	2288	2377	2322	156
			Weight gain (g)		
1st week	121	122	122	121	9.52
2nd week	244	244	251	250	19.5
3rd week	395	399	410	398	31.9
4th week	466	468	507	496	39.5
5th week	453	509	517	508	40.3
6th week	492	504	526	504	41
			Feed conversion ratio		
1st week	1.11	1.1	1.1	1.11	0.061
2nd week	1.49	1.48	1.45	1.47	0.081
3rd week	1.46	1.45	1.41	1.45	0.08
4th week	1.70	1.69	1.57	1.61	0.09
5th week	2.05	1.87	1.84	1.90	0.11
6th week	1.97	1.93	1.86	1.95	0.108

Table 3. Growth performance of Cobb 500 broilers supplemented with *Moringa stenopetala* leaf meal during the whole experimental period

Control, 0% of moringa leaf meal; MSL1, control + 1% moringa leaf meal; MSL1.5, control + 1.5% moringa leaf meal; MSL2, control + 2% moringa leaf meal. s.e.m., standard error of the mean

Parameter	Treatment				
	Control	MSL1	MSL1.5	MSL2	s.e.m.
Feed intake (g/day)	89.4	90.2	90.6	91.2	5.88
Initial bodyweight (g)	43.6	43.6	43.5	43.7	2.82
Final bodyweight (g)	2215	2288	2377	2322	154
Weight gain (g)	2171	2245	2333	2278	152
Feed conversion ratio	1.73	1.69	1.63	1.68	0.105

Table 4. Carcass characteristics and digestive organs of Cobb 500 broilers supplemented with *Moringa stenopetala* leaf meal

Control, 0% of moringa leaf meal. MSL1, control + 1% moringa leaf meal. MSL1.5, control + 1.5% moringa leaf meal. MSL2, control + 2% moringa leaf meal. s.e.m., standard error of the mean

Parameter	Treatment				
	Control	MSL1	MSL1.5	MSL2	s.e.m.
Gizzard weight (g)	43.6	44.6	45.6	45.2	2.89
Heart weight (g)	10	9.14	9.3	9.83	0.665
Liver weight (g)	42.7	46.8	48	50.2	2.83
Small intestine length (cm)	58.7	62.9	62.7	67.2	3.89
Small intestine weight (g)	54	57.5	54.4	60.6	3.58
Large intestine length (cm)	2.02c	2.51b	2.76ab	3.01a	0.134
Large intestine weight (g)	13.7	15.8	16.5	16.6	0.906
Proventriculus weight (g)	7.96	9.38	8.21	9.56	0.528
Pancreas weight (g)	3.08c	4.84b	5.15ab	5.61a	0.204
Spleen weight (g)	1.48	2.05	1.66	1.96	0.098
Duodenum length (cm)	31.7	36.5	34.5	35.3	2.1
Duodenum weight (g)	12.4	12.4	12.8	13.7	0.826
Jejunum length (cm)	80.7	82.2	74.2	86.7	5.36
Jejunum weight (g)	23.8ab	26.4ab	21.3b	27.5a	1.58
Ileum length (cm)	75.2	75.3	76.3	75.8	4.99
Ileum weight (g)	18.6	18.9	20	19.5	1.24
Head weight (g)	46.3	40	40.7	43.8	3.07
Legs weight (g)	66.7	66.9	68	67.8	4.43
Neck weight (g)	50.1	52.3	48	52	3.32
Liveweight (kg)	2.26	2.31	2.32	2.29	0.111
Hot carcass weight (kg)	1.59	1.68	1.78	1.67	0.085
Cold carcass weight (kg)	1.59	1.68	1.88	1.68	0.09
Breast meat weight (g)	467	483	528	475	25.3
Drumstick and thigh meat weight (g)	362	321	344	390	16.5
Wing meat weight (g)	147	139	143	149	6.84
Back meat weight (g)	244	239	212	233	10.2
Dressing yield weight (g)	1350	1270	1363	1304	65.4
Dressing (%)	70.7	73	76.7	73.3	3.67
pH	5.97	6.03	5.94	5.95	0.28
			Chemical composition of meat		
Moisture (%)	75	75.5	76	76	3.64
Total ash (%)	2.74b	2.89b	3.09ab	3.51a	0.148
Crude protein (%)	21.8	22.2	23.2	22.7	1.11
Ether extract (%)	3.06	3.05	3.04	3.08	0.145

control birds. Weight of pancreas of the supplemented birds was higher than that in the control group by 82%, 67% and 57% respectively, in MSL1, MSL1.5 and MSL2 groups. Jejunum weight of the control birds was not significantly different from that of the supplemented birds. However, the jejunum of birds in MSL1.5 was significantly lighter than that in MSL2.

Carcass traits

Table 4 shows the carcass traits of the study birds. Hot and cold carcass weights increased slightly (~0.19 kg), although not significantly, due to MSL supplementation. The dietary treatment did not significantly change the weight of breast meat, drumstick and thigh meat, wing meat and back meat (changes were <13%).

Dressing weight and dressing percentage tended to increase as a result of MSL supplementation; however, the increase was small and not significant. The meat of all birds had a similar pH, which was close to 6.

The chemical composition of meat in the current study is presented in Table 4. Mean moisture content of meat of the experimental groups ranged from 57% to 76%, without any significant variation. Crude protein of meat improved due to MSL supplementation, although the change was not significant. Ether extract of meat varied among the experimental groups, although not significantly. Only MSL2 had a significantly higher meat ash content by 28% than did the control.

Laying performance and egg quality

Table 5 presents laying performance and egg quality of layers as affected by the MSL supplementation. Supplementing layer diet by MSL did not affect feed intake, rate of lay egg mass and feed conversion ratio significantly ($P > 0.05$).

Concentrations of total cholesterol, triglyceride, low-density lipoprotein and high-density lipoprotein of blood were not significantly ($P > 0.05$) affected by the MSL supplementation.

Albumin weight, albumin height, yolk weight, yolk height and Haugh unit were not significantly affected by the MSL supplementation. Intensity of yolk colour increased significantly and linearly as a result of the increase in the level of MSL supplementation.

Exterior egg-quality parameters were slightly, but not significantly, affected by the MSL supplementation.

Antioxidant profile of plasma, meat and egg

Table 6 presents the effect of MSL supplementation on antioxidant profile of plasma, meat and eggs of the experimental animals. There was no significant effect of MSL supplementation on AOC, SOD, GPX and malonaldehyde of broilers at Day 21 and Day 42 of the trial. The concentration of malonaldehyde in breast and thigh meat after 2, 4 and 6 days of storage was not significantly affected by the MSL supplementation. Similarly, AOC, SOD and GPX of plasma of layers were not significantly influenced by the MSL supplementation.

Table 5. Laying performance and egg quality of Bovan Brown layers supplemented with *Moringa stenopetala* leaf meal

Control, 0% of moringa leaf meal; MSL1, control + 1% moringa leaf meal; MSL1.5, control + 1.5% moringa leaf meal; MSL2, control + 2% moringa leaf meal. s.e.m., standard error of the mean

Parameter	Control	MSL1	MSL1.5	MSL2	s.e.m.
Initial liveweight (g)	1427	1334	1430	1399	40.6
Final liveweight (g)	1645	1576	1665	1670	58.5
Feed intake (g/day)	103	99.3	99.5	99	3.43
Egg output (g/day)	57.2	57	57.4	58.1	1.87
Feed conversion ratio (g feed/g egg)	1.81	1.75	1.74	1.71	0.055
Rate of lay (%)	57.8	55.5	60.3	57.5	1.9
Blood lipid profile					
Total cholesterol (mg/dL)	126	121	118	117	10.9
Triglyceride (mg/dL)	115	101	94.5	111	4.16
Low-density lipoprotein (mg/dL)	264	241	258	270	8.7
High-density lipoprotein (mg/dL)	106	116	112	117	3.54
Internal egg quality					
Albumin weight (g/L)	36.5	36.2	36.1	37.2	1.99
Albumen height (mm)	7.87	7.5	7.9	7.5	0.241
Yolk weight (g/L)	14.1	14	14.3	14	0.465
Yolk height (mm)	17.7	17.2	17.2	16.8	0.58
Yolk colour	1d	4.75c	6.68b	7.41a	0.032
Haugh unit (%)	88.9	86.5	89.2	86.5	2.95
External egg quality					
Shell weight (g)	6.93	7.15	7.15	7.27	0.23
Shell strength (kg/cm^2)	3.8	3.54	3.48	3.86	0.127
Shell thickness (mm)	0.538	0.544	0.547	0.551	0.017
Specific gravity	1.06	1.07	1.07	1.08	0.034

Moreover, malonaldehyde concentration of yolk was not significantly affected by MSL.

Discussion

Identifying the nutritive value of feed is important to decide the inclusion level in livestock ration. Low variability in nutritive value of feed implies that the inclusion level of moringa leaf meal could be determined using tabulated values. Crude protein, EE and CF of *M. stenopetala* leaf were slightly different from the results of previous studies (Melesse *et al*. 2012, 2013), indicating a low variability in nutritive value. However, other abiotic factors might affect the nutritive value of *M. stenopetala* leaf, such as soil and age of the tree. Therefore, further studies on the variation in nutritive value of *M. stenopetala* leaf should be conducted before any final recommendation.

Heat stress is defined as a non-specific reaction to a combination of high temperature and humidity (Lara and Rostagno 2013). It has been reported that the upper boundary of the thermoneutral zone (the range of ambient temperature where the bird is able to maintain its core temperature by regulating dry-heat loss; Sonaiya and Swan 2004) for broilers is 29–30°C (Pereira and Nääs 2008). In the present

Table 6. Plasma antioxidant profile of egg and plasma of broilers and layers supplemented with *Moringa stenopetala* leaf meal

Control, 0% of moringa leaf meal; MSL1, control + 1% moringa leaf meal; MSL1.5, control + 1.5% moringa leaf meal; MSL2, control + 2% moringa leaf meal; AOC, total anti-oxidative capacity; SOD, total superoxide dismutase activity; GPX, glutathione peroxidase. s.e.m., standard error of the mean

Parameter	Control	MSL1	MSL1.5	MSL2	s.e.m.
Layer					
AOC (U/mL)	8.84	8.24	9.16	9.43	0.535
SOD (U/mL)	122	140	143	145	8.26
GPX (U/mL)	1399	1395	1456	1547	87
Broiler					
AOC (U/mL) 21 day	5.99	5.72	6.07	6.42	0.363
SOD (U/mL) 21 day	144	143	143	144	8.61
GPX(U/mL) 21 day	1329	1361	1456	1458	84.1
Malonaldehyde 21 day (nmol/mg protein)	5.55	5.77	5.22	5.66	0.333
AOC (U/mL) 42 day	7.77	7.93	8.91	7.65	0.484
SOD (U/mL) 42 day	133	139	139	135	8.18
GPX(U/mL) 42 days	1443	1385	1440	1444	85.7
Malonaldehyde 42 days (nmol/mg protein)	6.66	6.77	6.97	6.46	0.403
Malonaldehyde (nmol/mg protein)					
Yolk					
Fresh	2.23	2.25	2.36	2.33	0.137
15 days	2.45	2.42	2.44	2.25	0.143
20 days	2.33	2.31	2.36	2.3	0.139
30 days	3.37	3.35	3.34	3.33	0.201
Meat					
Breast meat, Day 2	0.987	1.02	0.923	0.889	0.057
Breast meat, Day 4	1.65	1.68	1.65	1.57	0.098
Breast meat, Day 6	1.76	1.92	1.78	1.63	0.106
Thigh meat, Day 2	1.13	1.13	1.12	1.19	0.069
Thigh meat, Day 4	1.38	1.27	1.24	1.21	0.077
Thigh meat, Day 6	1.44	1.39	1.34	1.29	0.082

study, the high temperature combined with high relative humidity were higher than the thermoneutral zone for broilers, which pinpoints a heat-stress status. Heat stress negatively affects broiler and layer performance by decreasing growth, egg production, increasing feed conversion ratio and increasing mortality rate (Lin *et al*. 2006; Yi *et al*. 2016). Furthermore, heat stress elevates accumulation of radicals in broiler meat and egg yolk, leading to a decline in shelf life.

The hypothesis of the present study was that MSL has a high concentration of glutamine, glutamic acid, vitamin and antioxidants; therefore, it might improve the production and quality of meat and eggs of chickens raised under heat stress. However, results of the present study showed that inclusion of MSL into broiler diet at a level up to 2% did not improve either performance or carcass traits, being in line with the findings of Nkukwana *et al*. (2014), Olugbemi *et al*. (2010) and Abou-Elezz Fouad Mohammed *et al*. (2012). Similarly, no positive effect of supplementing layers with MSL at a level up to 2% on laying performance and egg quality (except yolk colour) was found, which is in agreement with Lu *et al*. (2016). However, *Moringa* leaf supplementation was reported to have a positive effect on the performance of broilers (David *et al*. 2015; Khan *et al*. 2017) and layers (Olugbemi *et al*. 2010).

The exposure of MSL used in our study to ultraviolet rays during the 5 days of sundrying might have resulted in inactivation of significant amounts of antioxidants and vitamins, which are the compounds that have the potential of decreasing the negative consequences of heat stress in livestock (Ndawula *et al*. 2004; Potisate *et al*. 2014; Saini *et al*. 2014), while the majority of remnant antioxidants were used by the antioxidant defence systems of the birds to cope with heat stress.

The linear increase in the intensity of yolk colour in our study, corresponding to Akinola and Ovotu (2015), Teteh *et al*. (2016), Gakuya *et al*. (2014) and Lu *et al*. (2016), could be due to the increase in the pigment content in the diet (Pasaporte *et al*. 2014), which might not be affected by ultraviolet during sundrying. Most of the consumers in the world prefer eggs with yolk colour ranging from golden yellow to orange (Karunajeewa *et al*. 1984; Bejaei *et al*. 2011). Therefore, using MSL at a level of 1% in the layer diet would be recommended, so as to produce eggs with a higher preference by the consumers.

Heat stress stimulates oxidative stress, resulting in an increase in the production of reactive oxygen species and an increase in the cellular damage in many tissues and organs (Akhavan-Salamat and Ghasemi 2016). The body reacts to that by increasing the synthesis of antioxidant enzymes such as GPX and SOD, so as to reduce lipid peroxidation and cell damage (Huang *et al*. 2015). That would be reflected also in the increase in malonaldehyde, which is an index of lipid peroxidation and cellular damage (Ruberto and Baratta 2000). The current study showed that MSL supplementation at up to 2% did not improve antioxidant profile of plasma, oxidative stability of meat and egg yolk. This is in agreement with the broiler- and layer-performance results, suggesting a low antioxidant activity of MSL. This also implies that MSL supplementation would not improve shelf life of poultry meat and eggs.

Moringa oleifera leaf was reported to have high levels of β-sitosterol (90 mg/g), which has the ability to impede the absorption of cholesterol in the small intestine (Rajanandh and Kavitha 2010). However, supplementing layer diet with MSL in the current study did not significantly alter concentrations of triglyceride, total cholesterol, low-density lipoprotein and high-density lipoprotein in blood. It seems that MSL supplementation at a level up to 2% did not provide the layers with a high-enough amount of β-sitosterol to induce hypocholestemic activity.

The significant increase in pancreas weight suggests a presence of antinutritional activities due to MSL supplementation (Rocha *et al*. 2014). Accordingly, further analyses of moringa leaf antinutritional factors should be undertaken. The increase in the length of the large intestine, which is in agreement with Khan *et al*. (2017), might be attributed to the increase in the stay-time of ingesta due to the increase in CF content as a result of MSL supplementation (de Vries *et al*. 2012).

Conclusions

It is concluded that supplementing Cobb 500 broilers under hot and humid tropical conditions with sundried MSL at a level up to 2% did not improve growth performance, or carcass traits. Supplementing Bovan Brown layers with MSL at a minimum level of 1% increased the intensity of yolk colour, but did not alter laying performance and other interior and exterior egg traits.

The majority of chickens in the developing countries are indigenous. Thus, the use of MSL as a supplement to local chickens under hot and humid tropics should be studied.

The effect of the preservation method on the nutritive value of MSL should be studied when biomass yield of *Moringa* was reported to be generally high and exceeding the consumption in producing areas.

The results of the current study highlighted the importance of investigating concentration and biological activity of antinutritional compounds of MSL.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

The financial support of Jimma University College of Agriculture and Veterinary Medicines is highly appreciated. The technicians in Animal Nutrition and Post-harvest and Food science Technology Laboratory, Jimma University, are acknowledged for the chemical analysis of the experimental feeds. The support of Council of At-risk Academics provided to the corresponding author is acknowledged.

References

Abou-Elezz Fouad Mohammed K, Sarmiento-Franco L, Santos-Ricalde R, Solorio-Sanchez JF (2012) The nutritional effect of *Moringa oleifera* fresh leaves as feed supplement on Rhode Island Red hen egg production and quality. *Tropical Animal Health and Production* **44**, 1035–1040. doi:10.1007/s11250-011-0037-5

Abouelezz K, Mohammed F, Santos-Ricalde R, Solorio F (2011) Nutritional effects of dietary inclusion of *Leucaena leucocephala* and *Moringa oleifera* leaf meal on Rhode Island Red hens' performance. *Canadian Journal of Agricultural Science* **45**, 163–170.

Ahmad S, Khalique A, Pash T, Mehmood S, Hussain K, Ahmad S, Rasheed B, Awais M, Bhatti S (2017) Gıda katkısı olarak *Moringa oleifera* kabukları ile Beslemenin broiler tavuklarda performans ve kan parametreleri ile göğüs etinin kimyasal kompozisyonu ve biyoaktif bileşiklerine etkisi. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* **24**, 195–202. doi:10.9775/kvfd.2017.18616

Akhavan-Salamat H, Ghasemi HA (2016) Alleviation of chronic heat stress in broilers by dietary supplementation of betaine and turmeric rhizome powder: dynamics of performance, leukocyte profile, humoral immunity, and antioxidant status. *Tropical Animal Health and Production* **48**, 181–188. doi:10.1007/s11250-015-0941-1

Akinola L, Ovotu N (2018) Influence of *Moringa oleifera* leaf meal on egg lipids and blood constituents of laying hens *Journal of Experimental Agriculture International* **22**, 1–9. doi:10.9734/JEAI/2018/40432

Alebachew W, Tesfaye E, Tamir B (2016) Effects of feeding different dietary levels of *Moringa oleifera* leaf meal on egg production, fertility and hatchability of dual purpose Koekoek hens. *Middle East Journal of Scientific Research* **2**, 2909–2920. doi:10.5829/idosi.mejsr.2016.24.09.23907

AOAC (2006) 'Official methods of analysis.' (AOAC: Gaithersburg, MD, USA)

Baron R, Warren S (1997) Lipid abnormalities. 'Current medical diagnosis and treatment.' (Ed. S McPhee) pp. 1267–1276. (Appleton and Lange: Stanford, CA, USA)

Bejaei M, Wiseman K, Cheng KM (2011) Influences of demographic characteristics, attitudes, and preferences of consumers on table egg consumption in British Columbia, Canada. *Poultry Science* **90**, 1088–1095. doi:10.3382/ps.2010-01129

Card L, Nesheim MC (1972) 'Poultry production.' (Lea & Febiger: Philadelphia, PA, USA)

Cui Y, Wang J, Lu W, Zhang H, Wu S, Qi G (2018) Effect of dietary supplementation with Moringa oleifera leaf on performance, meat quality, and oxidative stability of meat in broilers. *Poultry Science* **97**, 2836–2844. doi:10.3382/ps/pey122

David LS, Vidanarachchi JK, Samarasinghe K, Cyril HW, Dematawewa CMB (2015) Effects of *Moringa* based feed additives on the growth performance and carcass quality of broiler chicken. *Tropical Agricultural Research* **24**, 12–20. doi:10.4038/tar.v24i1.7985

de Vries S, Pustjens AM, Schols HA, Hendriks WH, Gerrits WJJ (2012) Improving digestive utilization of fiber-rich feedstuffs in pigs and poultry by processing and enzyme technologies: a review. *Animal Feed Science and Technology* **178**, 123–138. doi:10.1016/j.anifeedsci.2012.10.004

Ferro-Vázquez C, Lang C, Kaal J, Stump D (2017) When is a terrace not a terrace? The importance of understanding landscape evolution in studies of terraced agriculture. *Journal of Environmental Management* **202**, 500–513. doi:10.1016/j.jenvman.2017.01.036

Gakuya DW, Mbugua PN, Mwaniki SM, Kiama SG, Muchemi GM, Njuguna A (2014) Effect of supplementation of *Moringa oleifera* (LAM) leaf meal in layer chicken feed. *International Journal of Poultry Science* **13**, 379–384. doi:10.3923/ijps.2014.379.384

Heuzé V, Tran G, Hassoun P, Bastianelli D, Lebas F (2019) Moringa (*Moringa oleifera*). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. Available at https://www.feedipedia.org/node/124 [Verified 9 May 2019]

Huang C, Jiao H, Song Z, Zhao J, Wang X, Lin H (2015) Heat stress impairs mitochondria functions and induces oxidative injury in broiler chickens. *Journal of Animal Science* **93**, 2144–2153. doi:10.2527/jas.2014-8739

Karthivashan G, Arulselvan P, Alimon AR, Safinar Ismail I, Fakurazi S (2015) Competing role of bioactive constituents in *Moringa oleifera* extract and conventional nutrition feed on the performance of Cobb 500 broilers. *BioMed Research International* **2015**, 1–13. doi:10.1155/2015/970398

Karunajeewa H, Hughes RJ, McDonald MW, Shenstone FS (1984) A review of factors influencing pigmentation of egg yolks. *World's Poultry Science Journal* **40**, 52–65. doi:10.1079/WPS19840006

Khan I, Zaneb H, Masood S, Yousaf MS, Rehman HF, Rehman H (2017) Effect of *Moringa oleifera* leaf powder supplementation on growth performance and intestinal morphology in broiler chickens. *Journal of Animal Physiology and Animal Nutrition* **101**, 114–121. doi:10.1111/jpn.12634

Lara LJ, Rostagno MH (2013) Impact of heat stress on poultry production. *Animals* **3**, 356–369. doi:10.3390/ani3020356

Lin H, Jiao HC, Buyse J, Decuypere E (2006) Strategies for preventing heat stress in poultry. *World's Poultry Science Journal* **62**, 71–86. doi:10.1079/WPS200585

Lu W, Wang J, Zhang HJ, Wu SG, Qi GH (2016) Evaluation of *Moringa oleifera* leaf in laying hens: effects on laying performance, egg quality, plasma biochemistry and organ histopathological indices. *Italian Journal of Animal Science* **15**, 658–665. doi:10.1080/1828051X.2016.1249967

Melesse A, Steingass H, Boguhn J, Schollenberger M, Rodehutscord M (2012) Effects of elevation and season on nutrient composition of leaves

and green pods of *Moringa stenopetala* and *Moringa oleifera*. *Agroforestry Systems* **86**, 505–518. doi:10.1007/s10457-012-9514-8

Melesse A, Getye Y, Berihun K, Banerjee S (2013) Effect of feeding graded levels of *Moringa stenopetala* leaf meal on growth performance, carcass traits and some serum biochemical parameters of Koekoek chickens. *Livestock Science* **157**, 498–505. doi:10.1016/j.livsci.2013.08.012

Mendieta-Araica B, Spörndly E, Reyes-Sánchez N, Salmerón-Miranda F, Halling M (2013) Biomass production and chemical composition of *Moringa oleifera* under different planting densities and levels of nitrogen fertilization. *Agroforestry Systems* **87**, 81–92. doi:10.1007/s10457-012-9525-5

Ndawula J, Kabasa JD, Byaruhanga YB (2004) Alterations in fruit and vegetable beta-carotene and vitamin C content caused by open-sun drying, visqueen-covered and polyethylene-covered solar-dryers. *African Health Sciences* **4**, 125–130.

Nkukwana TT, Muchenje V, Pieterse E, Masika PJ, Mabusela TP, Hoffman LC, Dzama K (2014) Effect of *Moringa oleifera* leaf meal on growth performance, apparent digestibility, digestive organ size and carcass yield in broiler chickens. *Livestock Science* **161**, 139–146. doi:10.1016/j.livsci.2014.01.001

NRC (1994) 'Nutrient requirements of poultry.' (National Academy Press: Washington, DC, USA). doi:10.1007/s10856-011-4313-8

Olugbemi TS, Mutayoba SK, Lekule FP (2010) Effect of moringa (*Moringa oleifera*) inclusion in cassava based diets fed to broiler chickens. *International Journal of Poultry Science* **9**, 363–367. doi:10.3923/ijps.2010.363.367

Pasaporte MS, Rabaya FJR, Toleco MM, Flores DM (2014) Xanthophyll content of selected vegetables commonly consumed in the Philippines and the effect of boiling. *Food Chemistry* **158**, 35–40. doi:10.1016/j.foodchem.2014.02.090

Pereira DF, Nääs IA (2008) Estimating the thermoneutral zone for broiler breeders using behavioral analysis. *Computers and Electronics in Agriculture* **62**, 2–7. doi:10.1016/j.compag.2007.09.001

Potisate Y, Phoungchandang S, Kerr WL (2014) The effects of predrying treatments and different drying methods on phytochemical compound retention and drying characteristics of moringa leaves (*Moringa oleifera* Lam.). *Drying Technology* **32**, 1970–1985. doi:10.1080/07373937.2014.926912

Rajanandh MG, Kavitha J (2010) Quantitative estimation of β-sitosterol, total phenolic and flavonoid compounds in the leaves of *Moringa oleifera*. *International Journal of Pharm Tech Research* **2**, 1409–1414.

Rama Rao SV, Raju MVLN, Prakash B, Rajkumar U, Reddy EPK (2019) Effect of supplementing moringa (*Moringa oleifera*) leaf meal and pomegranate (*Punica granatum*) peel meal on performance, carcass attributes, immune and antioxidant responses in broiler chickens. *Animal Production Science* **59**, 288–294. doi:10.1071/AN17390

Ramanathan L, Das NP (1992) Studies on the control of lipid oxidation in ground fish by some polyphenolic natural products. *Journal of Agricultural and Food Chemistry* **40**, 17–21. doi:10.1021/jf00013a004

Rocha C, Durau JF, Barrilli LNE, Dahlke F, Maiorka P, Maiorka A (2014) The effect of raw and roasted soybeans on intestinal health, diet digestibility, and pancreas weight of broilers. *Journal of Applied Poultry Research* **23**, 71–79. doi:10.3382/japr.2013-00829

Ruberto G, Baratta MT (2000) Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry* **69**, 167–174. doi:10.1016/S0308-8146(99)00247-2

Saini RK, Shetty NP, Prakash M, Giridhar P (2014) Effect of dehydration methods on retention of carotenoids, tocopherols, ascorbic acid and antioxidant activity in *Moringa oleifera* leaves and preparation of a RTE product. *Journal of Food Science and Technology* **51**, 2176–2182. doi:10.1007/s13197-014-1264-3

SAS (2012) 'SAS/STAT 12.1 user's guide.' (SAS Inc.: Cary, NC, USA)

Simainga S, Moreki J, Band F, Sakuya N (2011) Socioeconomic study of family poultry in Mongu and Kalabo districts of Zambia. *Livestock Research for Rural Development* **23**, 31

Sonaiya EB, Swan SEJ (2004) 'Small-scale poultry production.' (FAO: Rome, Italy)

Swain BK, Naik PK, Chakurkar E, Singh NP (2017) Effect of supplementation of *Moringa oleifera* leaf meal (MOLM) on the performance of Vanaraja laying hens management of mineral nutrition and associated stresses in Groundnut View project. *The Indian Journal of Animal Sciences* **87**, 353–355.

Tadelle D, Alemu Y, Peters KJ (2000) Indigenous chickens in Ethiopia: genetic potential and attempts at improvement. *World's Poultry Science Journal* **56**, 45–54. doi:10.1079/WPS20000005

Teteh A, Gbeassor M, Decuypere E, Tona K (2016) Effects of *Moringa oleifera* leaf on laying rate, egg quality and blood parameters. *International Journal of Poultry Science* **15**, 277–282. doi:10.3923/ijps.2016.277.282

Vyncke W (1970) Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity *Fette, Seifen. Anstrichmittel* **72**, 1084–1087. doi:10.1002/lipi.19700721218

Wang J, Zhang HJ, Samuel KG, Long C, Wu SG, Yue HY, Sun LL, Qi GH (2015) Effects of dietary pyrroloquinoline quinone disodium on growth, carcass characteristics, redox status, and mitochondria metabolism in broilers. *Poultry Science* **94**, 215–225. doi:10.3382/ps/peu050

Webster A (1983) 'Nutrition and the thermal environment in nutritional physiology of farm animals.' (Eds J Rook and P Thomas) (Longman: New York, NY, USA)

Wiseman J (1987) 'Feeding of non-ruminant livestock.' (Butterworth and Co.: Nottingham, UK)

Yi D, Hou Y, Tan L, Liao M, Xie J, Wang L, Ding B, Yang Y, Gong J (2016) N-acetylcysteine improves the growth performance and intestinal function in the heat-stressed broilers. *Animal Feed Science and Technology* **220**, 83–92. doi:10.1016/j.anifeedsci.2016.07.014

Zhang L, Yue HY, Wu SG, Xu L, Zhang HJ, Yan HJ, Cao YL, Gong YS, Qi GH (2010) Transport stress in broilers. II. Superoxide production, adenosine phosphate concentrations, and mRNA levels of avian uncoupling protein, avian adenine nucleotide translocator, and avian peroxisome proliferator-activated receptor-γ coactivator-1α in skele. *Poultry Science* **89**, 393–400. doi:10.3382/ps.2009-00281

Handling editor: Konstantinos Mountzouris