Effects of feeding diets with processed *Moringa oleifera* stem meal on growth and laying performance, and immunological and antioxidant activities in laying ducks

S. L. Yang,^{*,†,1} R. C. Yang,^{*} X. Zhou,^{*} S. H. Yang,^{*} L. L. Luo,^{*} Y. C. Zhu,^{*} and Surintorn Boonanuntan[‡]

*College of Animal Science, Guizhou University, Guiyang, 550025, Guizhou, China; [†]Guiyang Muren Agricultural Science and Technology Development Co. Ltd., Guiyang, 550600, Guizhou, China; and [‡]School of Animal Production and innovation, Suranaree University of Technology, Nakhon Rachasima, 30000, Thailand

ABSTRACT This study was conducted to determine the effect of *Moringa oleifera* stem (**MOS**) meal in ducks. A total of 225 ducklings at 1 D of age were randomly assigned to 3 dietary treatment groups with 3 replicates of 25 each. The growth experiment lasted 63 D. The egg experiment started from 23 to 27 wk of age. Ducks were randomly divided into 3 treatment groups with 3 replications of 15 each. The following dietary treatments were applied: 1) Control (CON), basal diet + 0% MOS meal; 2) basal diet + 2% MOS meal; 3) basal diet + 4% MOS meal. During 0 to 4 wk of age, ducks fed 2% MOS diet showed significantly increase in average daily feed intake (ADFI) and average daily gain (ADG; P < 0.05) and ducks fed 4% MOS diet had a significant improvement in feed conversion rate (FCR; P < 0.05). However, ADFI, ADG, and FCR were not affected significantly during 5 to 9 wk of age (P > 0.05). In egg production experiment, ADFI, average egg weight, laying rate, and FCR showed significant increase in 4% MOS diets

(P < 0.05). Laying ducks fed 4% MOS diet had a higher egg shape index, whereas a lower yolk color compared with CON (P < 0.05). The proportion of broken shell eggs were zero in experimental diets, whereas 3% of which occurred in CON (P < 0.05). However, no significant effects in proportion of soft shell eggs, proportion of abnormal-shape eggs, albumen height, haugh unit, and eggshell thickness were observed among all treatments (P > 0.05). For serum biochemical parameters, total protein and albumin were increased in MOS diets during 0 to 4 wk of age, but decreased during 5 to 9 wk of age. For serum antioxidant index, superoxide dismutase and glutathione peroxidase values were increased whereas malondialdehyde values were decreased in MOS diets from 0 to 9 wk of age. The results suggest that MOS positively affects early growth performance and laying performane of duckling but partially affects egg quality. The antioxidative activity and immunological index may be improved.

Key words: Moringa oleifera stem, laying performance, immunological index, antioxidant activities

2020 Poultry Science 99:3445–3451 https://doi.org/10.1016/j.psj.2020.04.002

INTRODUCTION

Moringa oleifera tree (Moringa) is a popular multipurpose tree, naturally cultivated in tropical and subtropical countries due to its considerable inherent nutritional, antioxidant, and phytochemical benefits, as well as its ability to survive in diverse climatic conditions (Shah et al., 2016). Research on the use of various parts of the *M. oleifera* Lam. plant as a nutritional and nutraceutical resource for human and animal diets has increased in recent years. Several parts of the moringa tree like leaves and pods are rich in the number of vital nutrients (Babiker et al., 2017). Other parts of the tree including roots have various medical applications (Shah et al., 2016). The application of M. oleifera in livestock feed as a source of protein, antibiotic, and antioxidant compounds has been reported in the literature. Results showed that M. oleifera may improve growth performance in rabbits, meat organoleptic quality in pigs, as well as reduce milk yield but without changing the composition and the organoleptic characteristics of milk, and the rate of microbial growth in meat products after processing and cold storage (Mendieta-Araica et al., 2011; Adeniji and Lawal, 2012; Mukumbo et al., 2014).

^{© 2020} Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Received October 23, 2019.

Accepted April 1, 2020.

¹Corresponding author: Shenglinyang@126.com

The main part of *M. oleifera* utilized as feed resource is its leaf because it is readily eaten by animals. Recent reports of the analyzed nutrient composition of the leaves, seeds, and stems of the plant show that they are rich in protein, essential amino acids, minerals, vitamins, and other bioactive compounds (Valdez-Solana et al., 2015). Data on the nutrient composition of the roots are still scarce. On dry matter basis, the crude protein content of *M. oleifera* leaf has been reported to be 10.74 to 30.29 g/100 g. Crude fiber ranges from 7.09 to 35.0 g/100 g, fat 6.50 to 20.00 g/100 g, and ash 7.64 to 10.71 g/100 g. The nutritional analysis of M. oleifera seeds revealed that they contain about 9.98 to 51.80 g/100 g crude protein, 17.26 to 20.00 g/100 g crude fiber, 38.67 to 43.60 g/100 g fat, and 3.60 to 5.00 g/100 g ash. Compared with leaves and seeds, M. oleifera stem (MOS) displays 12.77 g/100 g crude protein, 2.0 g/100g fat, 78.58 g/100 g nitrogen free extract, and 6.65 g/ 100 g ash (Shih et al., 2011). The substantial variation in the nutritional composition may be due to factors such as growth environment, stage of harvest, soil type, and method of processing.

In poultry, several studies indicated that M. oleifera leaf meal can be used as a protein source in poultry diets without causing any adverse effects on growth performance (Makanjuola et al., 2014; Onunkwo and George 2015). Reports on the immune responses of broiler chickens fed M. oleifera seeds and leaves showed that they can increase the production of red blood cells, white blood cells, and the hemoglobin level in the blood system (Stevens et al., 2015). Ahmad et al. (2017) reported that M. oleifera pod meal supplementation affects egg mass, serum biochemistry, and bioactive compounds of the egg volk positively in HyLine W36 layer. In fact, MOS has not been fully applied in the past on account of its high fiber content. To the authors' knowledge, a few studies until now have been conducted in which M. olei*fera* products are utilized as feed resource, especially in laying birds. This study aimed to evaluate the effects of feeding diets with processed MOS meal on growth and laying performance, as well as immunological and antioxidant activities in laying ducks.

MATERIALS AND METHODS

MOS Meal, Ducks, Feeding, and Experimental Design

The Animal Welfare Committee of Guizhou University (Guiyang, Guizhou, China) approved the animal care protocol used for these experiments. *M. oleifera* stem meal was bought from Kunming Qoaoshanji Food Co. Ltd., China. *M. oleifera* stem was obtained from dry *M. oleifera* tree after eliminating all leaves and processed by air drying and ground into stem meal through a 0.425-mm sieve. The main chemical composition included 12.1% of crude protein, 2.4% of fat, and 29.3% of crude fiber.

A total of 225 Sansui ducklings at 1 D of age with an average initial body weight of 41.01 ± 4.51 g were placed

in galvanized wire cages (0.98 m^2) with 25 birds per cage. The cages were equipped with feeder, nipple drinker, and raised plastic floors. All ducks were housed in an environmentally controlled facility. This 63 D experiment consisted of 3 treatments with 3 replications (cages) per treatment and 25 ducks per cage in a randomized complete block design. For the egg production experiment, when ducks were fed to 120 D of age, eggs could be observed in all cages, 135 female ducks from all 225 ducks used in the former experiment were selected to be fed in the cages for laying eggs; the experiment was started when the age ranged from 23 to 27 wk. The experiment included 3 treatments with 3 replications, and each treatment was performed on 15 ducks.

A 3-phase feeding program was used: a starter diet from 0 to 4 wk, a grower diet from 5 to 9 wk, and diets of the laying period (23–27 wk). Three basal diets (Table 1) were formulated to meet or exceed the NRC (1998) requirements for ducks, and the dietary treatments were: 1) control, basal diet without adding MOS meal; 2) basal diet + 2% MOS meal (20 g MOS/kg diet); and 3) basal diet + 4% MOS meal (40 g MOS/ kg diet). Diets were fed in powdery form and feed and water were provided ad libitum throughout the experiment. The environmental temperatures were set as follows: for the first week the temperature was 33°C, thereafter it was reduced 1°C each week from the second week until the fourth week, and from the fifth to ninth weeks and 23^{th} to 27^{th} wk, the temperature was kept at 25°C to 28°C until the experiments came to an end. The relative humidity was maintained at 65 to 70%throughout the whole experimental period.

Sampling and Measurements

For duck growth performance, the ducks were weighed once a week and feed intake was recorded daily in the morning. The average daily gain (**ADG**) and feed conversion rate (**FCR**) could be calculated after the experiments were completed. Mortality was recorded as it occurred, and the weights of dead birds were used to adjust feed intake to weight gain ratio.

For laying performance, FCR, the average daily feed intake (**ADFI**) was calculated using weekly recorded values. Egg production was recorded daily per cage, and the laying rates (**LR**) were calculated at the end of the laying experiment. The average egg weight (**AEW**) was determined by dividing the total weight of the collected eggs by the number of eggs laid per replicate. FCR was calculated based on feed intake and egg production data.

To evaluate the egg quality traits, over the experimental period all eggs from each replicate were collected, identified, and evaluated for the following characteristics as described previously (Hammershøj and Steenfeldt, 2012): albumen height (AH), Haugh unit (HU), egg shape index (ESI), yolk color (YC), eggshell thickness (ET), and eggshell strength (ES). Proportion of soft shell eggs (PSSE), proportion of broken shell (PBS), and proportion of abnormal-shape eggs (PASE) could be

Table 1. Ingredients and chemical composition of diets with or without Moringa oleifera stem meal.

	Starter $(0-4 \text{ wk})$			Grower (5–9 wk)			Laying period (23–27 wk		
Items	$\operatorname{CON1}^1$	MOS11	MOS12	CON2	MOS21	MOS22	CON3	MOS31	MOS33
Ingredients (%)									
Corn	63.85	62.85	61.85	64.85	63.85	62.85	55.75	54.75	53.75
Soybean meal	27.83	26.83	25.83	25.83	24.83	23.83	27.40	26.40	25.40
Wheat bran	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Rapeseed cake	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
$CaHPO_4$	1.50	1.50	1.50	2.50	2.50	2.50	2.75	2.75	2.75
Limestone	0.85	0.85	0.85	0.85	0.85	0.85	7.25	7.25	7.25
NaCl	0.25	0.25	0.25	0.25	0.25	0.25	0.35	0.35	0.35
Premix ²	0.22	0.22	0.22	0.22	0.22	0.22	1.00	1.00	1.00
M. oleifera stem meal	0.00	2.00	4.00	0.00	2.00	4.00	0.00	2.00	4.00
Analytical composition ³									
Crude protein (%)	19.31	19.00	18.75	18.52	18.24	17.95	18.40	18.15	17.87
Metabolizable energy	11.44	11.34	11.25	11.16	11.32	11.23	10.06	10.22	10.12
(MJ/kg)									
Calcium (%)	0.96	0.96	0.96	1.26	1.25	1.25	3.61	3.61	3.60
Available phosphorus (%)	0.43	0.43	0.42	0.66	0.65	0.65	0.71	0.71	0.71
Lysine (%)	1.04	1.00	0.98	0.99	0.96	0.93	1.00	0.97	0.94
Methionine (%)	0.29	0.28	0.27	0.28	0.27	0.26	0.27	0.27	0.26

Abbreviations: CON, control; MOS, Moringa oleifera stem.

 1 CON1: control diet for the duckling at 0 to 4 wk of age; CON2: control diet for the grower ducks at 5 to 9 wk of age; CON3: control diet for the laying ducks at 23 to 27 wk of age.

²Premix contents (per kg): Cu 10 mg; Fe 80 mg; Mn 60 mg; Zn 60 mg; I 0.4 mg; Se 0.2 mg; VA 4,000 IU; VE 20 mg; VK 32 mg; VB1 3.5 mg; niacin 50 mg; folic acid 1.0 mg; calcium pantothenate 10 mg; pyridoxol 2.5 mg; VB12 0.01 mg; biotin 0.1 mg.

³Calculated values.

counted after the experiment came to an end. For the analyses of quality, AH and egg weight data were utilized in the calculation of HU by the following equation: $HU = 100 \log (H + 7.57 - 1.7 W0.37)$. ESI values were computed using the vertical diameters and transverse diameters of eggs measured by vernier caliper based on the equation: ESI = transverse diameter/vertical diameter. YC was analyzed by using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd., Osaka, Japan), where the L^{*}, a^{*}, and b^{*} values were recorded and reflect lightness (0 = black, 100 = white), redness (-100 = green), 100 = red, and yellowness (-100 = blue,100 = yellow), respectively. ET was regarded as the average of the thicknesses obtained in the 3 eggshell regions (apical, equatorial, and basal), using a digital micrometer with 0.01 mm divisions. For the ES values, before analysis, individual egg weights were recorded. The ES value of each egg was analyzed by uniaxial compression at the equator of the egg (Hammershøj and Steenfeldt, 2012). The force and displacement data at the shell fracture point were recorded giving the shell strength (N) and shell elasticity or shell fracture point (m). The shell modulus (N/mm) was per definition calculated as the slope of the initial part (0.01-0.03 mm) of the force-displacement curve relating to the stiffness of the egg shell.

At the end of the experiment (at D 28 and 63 of age), 10 birds from each replicate were randomly selected from each cage and 5 mL of blood sample from each bird were collected from the jugular vein into a sterile syringe and stored at -4° C. Blood samples were then centrifuged at 3,000 \times g for 15 min and serum was separated. For serum biochemical parameters, total protein (**TP**), albumin (**ALB**), IgG, IgA, and IgM, were analyzed using an automatic biochemical analyzer (Hitachi 7180, Japan). For serum antioxidant index, the levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) were analyzed using ELISA method (Jiancheng Biotechnology Institute, Nanjing, China) following the kit instructions (Yan et al., 2019).

Statistical Analyses

Data were analyzed using one-way ANOVA by using SPSS 19.0 (SPSS software for Windows; SPSS Inc., Chicago, IL). The results were indicated as means \pm SEM. Duncan's test was used for multiple comparisons. Significance was declared at P < 0.05.

RESULTS

Growth Performance

As shown in Table 2, during 0 to 4 wk of age, ducks fed 2% MOS meal had a significant increase in ADFI (P < 0.05), whereas no differences were detected in birds fed 4% MOS compared to birds fed control diet (P > 0.05). ADG increased significantly when ducks were fed 2% MOS and 4% MOS compared to the control diet. No significant difference in FCR between the control and 2% MOS (P > 0.05) was detected in spite of a slight improvement in birds fed 2% MOS. However, there was significant improvement in FCR in birds fed 4% MOS compared to birds fed without MOS (P < 0.05). Interestingly, there were no differences in ADFI, ADG, and FCR among ducks fed control diet, 2% MOS, and 4% MOS during 5 to 9 wk of age (P > 0.05).

Laying Performance

As presented in Table 3, during the laying period of the experiment (23–27 wk), ADFI showed a significant

reduction in ducks fed 2% MOS (P < 0.05), but a significant increase in birds fed 4% MOS (P < 0.05) compared to the control. AEW displayed higher values in ducks fed both 2% MOS and 4% MOS, but no differences were observed between birds fed 2% MOS and birds fed 4% MOS (P > 0.05).

For LR, ducks fed 4% MOS had higher LR value (69%), whereas birds fed 2% MOS showed a lower LR (42%) compared to the control (61%) (P < 0.05). A similar result was observed in FCR values; ducks fed 4% MOS had higher FCR value (3.75:1), whereas ducks fed 2% MOS displayed a lower FCR value (5.07:1) compared to the control (4.44:1) (P < 0.05).

Egg Quality

To evaluate the egg quality, we included some statistical indices such as PSSE, PBS, and PASE besides AH, HU, ESI, and so on. As listed in Table 3, no significant differences were observed in PSSE and PASE between the control group and the ducks fed the experimental diets (2% MOS and 4% MOS) (P > 0.05); however, a significant difference in PBS between the control diet (3%)and birds fed the experimental diets (0%) was detected (P < 0.05), but there was no significant difference between ducks fed 2% MOS and ducks fed 4% MOS (P > 0.05). There were no differences in AH, HU, and ET between the control group and ducks fed 2% MOS and 4% MOS (P > 0.05). However, ducks fed 4% MOS had a higher ESI (1.36) than the control (1.32) (P < 0.05), but no significant difference between ducks fed 2%MOS and ducks fed 4% MOS was observed (P > 0.05). Ducks fed 4% MOS had a lower YC (6.56) than the control (7.83) (P < 0.05), but no significant difference between ducks fed 2% MOS and ducks fed 4% MOS was observed (P > 0.05). For ES (ET), ducks fed 2% MOS displayed a higher ET value than the control, but there was no significant difference between control and ducks fed 4% MOS, and between birds fed 2% MOS and birds fed 4% MOS.

Serum Biochemical Indices

The effect of dietary *M. oleifera* supplementation on serum biochemical parameters is displayed in Table 2. No significant effect on serum TP was observed in the group of 2% MOS diet (P > 0.05); however, a significant increase in the group of 4% MOS diet (P < 0.05) was noted compared to the control during the starter period (0-4 wk). The TP values significantly decreased in 2%MOS diet (P < 0.05) and slightly decreased in 4% MOS diet (P > 0.05) compared to the control during the grower period (5–9 wk). Interestingly, serum ALB concentrations significantly increased in 2% MOS diet and in 4% MOS diet (P < 0.05) compared to the control during the starter period (0-4 wk), whereas significant reductions were detected in 2% MOS diet and 4%MOS diet (P < 0.05) compared to the control during the grower period (5–9 wk). No significant effects on IgG, IgA, and IgM were noted among all the treatments (P > 0.05) during the starter period (0–4 wk) and the grower period (5–9 wk), except for a significant increase in IgA in 2% MOS diet (P < 0.05) and significant increase in IgG in 2% MOS diet and 4% MOS diet (P < 0.05).

Serum Antioxidant Status

As presented in Table 2, no significant effects on SOD were detected among all treatments (P > 0.05) during the starter period (0–4 wk). However, a slight decrease in SOD in 2% MOS diet and a slight increase in 4%MOS diet could be detected (P > 0.05) during the grower period (5–9 wk), while the SOD value in 4%MOS diet was significantly higher than that in 2%MOS diet (P < 0.05). MDA values decreased significantly in both 2% MOS diet and 4% MOS diet (P < 0.05) compared to the control, whereas no significant differences between 2% MOS diet and 4% MOS diet were noted (P > 0.05) during the starter period (0–4 wk), only the MDA value in 4% MOS diet was significantly lower than the control (P < 0.05) during the grower period (5–9 wk), and no significant effects were observed among the other treatments (P > 0.05). For GSH-Px activity, the 2% MOS diet group displayed an improvement, whereas the 4% MOS diet group showed a reduction compared with the control group, but no significant differences were noted (P > 0.05) during the starter period (0-4 wk). There were improvements in GSH-Px value in both 2% MOS diet and 4%MOS diet but the highest value was recorded in 2%MOS diet (P < 0.05), and no significance between 2% MOS diet and 4% MOS diet was detected (P > 0.05) during the grower period (5–9 wk).

DISCUSSION

To the authors' knowledge, no studies until now have been reported on the effects of MOS on growth performance and egg production in laying ducks. However, reports concerning M. oleifera products on growth performance in broiler chickens and egg production in laying hens are available easily. Alabi et al. (2017) applied aqueous M. oleifera leaf extracts to study the growth performance of broiler chickens; the results demonstrate that ADG was higher in extractsupplemented groups than the control. Feed intake was highest in birds on positive control. FCR was lower in extracts fed groups. Abdulsalam et al. (2015) found that supplemented diets with Moringa leaf meal in broilers could enhance the growth performance in the finisher period. Similarly, inclusion of *M. oleifera* leaves at higher levels (15 and 20%) in broiler diets resulted in a higher growth rate and better health status in broilers (Alnidawi et al. 2016). In the current study, ADG and FCR were higher in ducks fed 2% MOS diet and 4%MOS diet than the control during the starter period (0–4 wk), but no significant effects were detected during the grower period (5–9 wk). ADFI was higher in ducks fed 2% MOS diet and 4% MOS diet, but no significance

Table 2. Effect of *Moringa oleifera* stem powder added in diets of growing laying ducks.

	Starter (0–4 wk)					Grower (5–9 wk)								
	Cont	trol	2% l	MOS	4% MOS	3	<i>P</i> -value	Cor	ntrol	2%]	MOS	4% M	MOS	<i>P</i> -value
Growth performance	a t tab		2= 442		o t t ob		0.000	00.01						0.010
ADFI (g)	34.49	± 0.73	37.44^{a}	± 1.19	$34.10^{\circ} \pm$	0.69	0.003	82.81	± 1.74	84.22	± 2.68	83.97	± 1.70	0.910
ADG (g)	11.83°	± 0.18	13.23	± 0.59	$14.00^{a} \pm$	0.34	0.001	19.28	± 1.11	19.23	± 0.52	20.48	± 0.39	0.367
FCR	2.91^{a}	± 0.07	2.84^{a}	± 0.20	$2.44^{\circ} \pm$	0.01	0.001	4.30	± 0.27	4.38	± 0.04	4.10	± 0.12	0.497
parameters														
TP (g/L)	29.39^{b}	± 3.27	$33.82^{\mathrm{a,b}}$	2 ± 2.19	$37.68^{\rm a}$ ±	5.44	0.048	34.99^{a}	± 8.44	31.19^{b}	± 5.70	$32.30^{\mathrm{a,b}}$	± 3.83	0.064
ALB(g/L)	10.55^{b}	± 0.32	$14.68^{\rm a}$	± 1.73	$15.77^{a} \pm$	0.16	0.001	22.65^{a}	± 1.75	19.72^{b}	± 1.48	$19.80^{ m b}$	± 1.14	0.001
IgG (g/L)	1.27	± 0.01	1.28	± 0.01	$1.27 \pm$	0.01	0.603	1.43^{b}	± 0.02	1.44^{a}	± 0.01	1.44^{a}	± 0.01	0.285
IgA(g/L)	1.36^{b}	± 0.03	1.39^{a}	± 0.02	$1.38^{\mathrm{a,b}} \pm$	0.02	0.078	1.26	± 0.01	1.25	± 0.03	1.26	± 0.01	0.146
IgM (g/L)	1.26	± 0.01	1.28	± 0.01	$1.27 \pm$	0.01	0.598	1.22	± 0.02	1.21	± 0.02	1.21	± 0.02	0.001
Serum antioxidant														
index														
SOD (U/mL)	42.4	± 3.78	47.36	± 2.04	$47.25 \pm$	3.32	0.411	$27.87^{a,t}$	2 ± 2.35	25.02^{b}	± 2.60	28.87^{a}	± 3.38	0.003
MDA (nmol/mL)	7.66^{a}	± 2.75	5.12^{b}	± 1.19	$5.51^{b} \pm$	1.53^{b}	0.049	7.43^{a}	± 1.27	$6.47^{a,l}$	$^{\circ} \pm 1.34$	$5.89^{ m b}$	± 0.58	0.032
$\operatorname{GSH-Px}\left(\mathrm{U/mL} ight)$	$283.53^{\mathrm{a,b}}$	± 16.73	308.09^{a}	± 10.78	$267.63^{\rm b} \pm 2$	8.86	0.015	277.33 ^b	\pm 25.75	315.00^{a}	± 14.91	297.73 ^{a,b}	± 23.53	0.059

^{a-c} Mean \pm SE values with different superscripts in the same row differ (P < 0.05); mean \pm SE values with the same superscripts or without superscripts in the same row differ (P > 0.05).

Abbreviations: ADFI, average daily feed intake; ADG, average daily body weight gain; ALB, albumin; FCR, feed conversion rate; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; MOS, *Moringa oleifera* stem; SOD, superoxide dismutase; TP, total protein.

was observable during the whole period (0–9 wk) (Table 2). These results are consistent with Alabi et al. (2017), Abdulsalam et al. (2015), and Alnidawi et al. (2016). The higher ADG and FCR values in this study might be related to the presence of different bioactive components in moringa stem that may play a role in improved nutrient utilization in supplemented birds (Mahfuz and Piao. 2019).

In contrast, our results are not consistent with those of Gakuya et al. (2014) and Onunkwo and George (2015), who found that feeding moringa leaf meal in broilers led to a lower feed intake with higher FCR, which was due to the presence of anti-nutritional factors in moringa leaves used in the experiment diets as row basis. Similarly, Gadzirayi et al. (2012) used *M. oleifera* leaf meal

as supplementation of conventional soybean meal in broiler diets at 0, 25, 50, 75, and 100% level. The authors did not find any significant differences in feed intake and body weight gain between control and 25% level of moringa supplementation.

Serum biochemical parameters provide useful information for the evaluation of the health status of birds and reflect many metabolic alterations of organs and tissues (Makanjuola et al., 2014). SOD and GSH-Px as key enzymes of the antioxidant system play a crucial role in eliminating free radicals, reducing oxidative damage, and maintaining cell structure. The activities of SOD and GSH-Px in the serum were decreased, whereas the level of MDA increased under oxidative stress (He et al., 2016). *M. oleifera* is known to be a potential

Table 3. Effect of Moringa oleifera stem meal added in diets of laying ducks(23-27 wk).

	Diets						
	Control	2% MOS	4% MOS	<i>P</i> -value			
Laying performance ADFI (g) AEW (g) LR FCR Egg quality PSSE PBS PASE AH (mm) HU FSI	$189.04^{b} \pm 0.71$ $52.81^{b} \pm 5.12$ $0.61^{b} \pm 0.01$ $4.44^{b} \pm 0.08$ 0.01 ± 0.00 $0.03^{a} \pm 0.00$ 0.00 ± 0.00 5.17 ± 2.00 67.04 ± 19.66 $1.22^{b} \pm 0.05$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{c} 0.001\\ 0.001\\ 0.001\\ 0.001\\ 0.147\\ 0.02\\ 0.655\\ 0.995\\ 0.989\\ 0.016\end{array}$			
$\begin{array}{c} \text{ESI} \\ \text{YC} \\ \text{ES} (\text{N/m}^2) \\ \text{ET (mm)} \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{c} 0.010 \\ 0.012 \\ 0.158 \\ 0.449 \end{array}$			

 $^{\rm a-c}$ Mean \pm SE values with different superscripts in the same row differ (P < 0.05); mean \pm SE values with the same superscripts or without superscripts in the same row differ (P > 0.05).

Abbreviations: ADFI, average daily feed intake; AEW, average egg weight; AH, albumen height; ES, eggshell strength; ESI, egg shape index; ET, eggshell thickness; FCR, feed conversion rate; HU, haugh unit; LR, laying rate; MOS, *Moringa oleifera* stem; PASE, proportion of abnormal-shape eggs; PBS, proportion of broken shell; PSSE, proportion of soft shell eggs; YC, yolk color.

antioxidant with some antioxidant properties due to the presence of vitamins C and E, carotenoids, flavonoids, and selenium (Moyo et al. 2016). *M. oleifera* leaves contain various phytochemicals (carotenoids, flavonoids, chlorophyll, phenolics, xanthines, cytokines, alkaloids, etc.) that might have a role in improving health status (Falowo et al. 2014). In the current study, ducks fed MOS supplemented diets showed increased serum SOD, GSH-Px, and decreased MDA, which implied that MOS (2 or 4%) can exert potent antioxidative activity in Sansui laying ducks (Table 2). The probable explanation for this may be due to the antioxidant properties of phytochemicals in the Moringa stems.

Serum immunoglobulin and complement components are usually used to evaluate the immune status of hens due to their important roles in immune function. M. oleifera leaf extracts have been found to exhibit both immunosuppressive and immunostimulatory activities (Rachmawati et al., 2014). The immunomodulatory effect of leaves is mediated through reduction in cyclophosphamide induced immunosuppression by stimulating both cellular and humoral immunity (Gupta et al., 2010), which is attributed to the presence of compounds like isothiocyanates and glycoside cyanides (Sudha et al., 2010). Effects of Chinese herbal medicines like coriander seed, Coptis chinensis, rosemary etc. on immunological index (IgM, IgG, and IgA) have been largely reported, which displayed improvements at different levels in the immunological index when adding herbs in diets (Hosseinzadeh et al. 2014; Alagawany et al., 2015; Yang et al. 2019). Nonetheless, little is known about the effects of M. oleifera products on immunological index in birds. In the present study, ducks fed 2% MOS diet and 4% MOS diet had higher TP and higher ALB during the starter period (0–4 wk), slightly higher IgM, IgG, and IgA values were also noted during the starter period (0-4 wk), suggesting that MOS meal may play a role in improving the immunological index. However, no significant effects could be observed during the grower period (5–9 wk). Further experiments are necessary to demonstrate what proportions of MOS meal in diets are appropriate.

In the laying period, the experiment was conducted from 23 to 27 wk; ducks fed 4% MOS diet had higher ADFI, AEW, LR, and FCR than the control. This result is almost in line with the report by Moreki and Gabanakgosi (2014), except that laying hens were applied in their experiment and lower feed intake occurred. They concluded that higher egg production may be related to improved digestibility in the supplemented groups due to different active components in moringa leaves. Interestingly, moringa stem meal was used in our study, whose fiber content is high, but a positive result in egg production was observed. We concluded that higher ADFI, AEW, LR, and FCR may be associated with the presence of different bioactive components in moringa stem. Further experiments must to be conducted to confirm if bioactive components are present in moringa stem or not, and the experiments need to be designed to verify if the above result is positive or negative.

Egg quality was recorded in Table 3; no changes in PSSE and PASE among all treatments were observed. However, PBS was 3% in the control group and zero in ducks fed MOS groups. Accordingly, ES values were significantly higher in 2% MOS diet and 4% MOS diet than the control. This suggests that PBS may be closely related to ES. ES is an important parameter in the poultry industry; economic loss caused in the process of transportation and storage could be reduced when the value of ES is high enough. In our study, increased ES and decreased PBS may be associated with abundant calcium (780 mg/100 g) in moringa stem (Shih et al., 2011). Recent studies have shown that the inclusion of M. oleifera leaf powder in poultry diets improved the production and quality of eggs in laying hens (Gakuya et al., 2014; Lu et al., 2016). The inclusion of 2.5 and 5% of *M. oleifera* leaf powder in layer diet improved the egg number per week, egg weight, egg width, egg surface, yolk weight, yolk height, albumen weight, and volk ratio when compared to the control diet (Ebenebe et al., 2013). In the current study, AEW, AH, and ESI in 2% MOS diet and 4% MOS diet were higher than the control, which are in agreement with the results of Ebenebe et al. (2013), Gakuya et al. (2014), and Lu et al. (2016). However, YC scores in 2% MOS diet and 4% MOS diet were significantly lower than the control. The decreased YC scores could be due to the low carotene content in moringa stems. No significant differences in PASE, HU, and ET among all treatments were observed in this study.

Thus, we concluded that MOS positively affects early growth performance of ducklings and laying performance, and also affects partially egg qualities such as PBS, ESI, and ES. *M. oleifera* stem can exert potent antioxidative activity and play a role in improving the immunological index in laying ducks.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (No. 31960682), Special Project for Chinese Government Support Local Government (No. [2019]4021), and Agricultural Major Special Project of Guizhou Province (No. [2019]5203).

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

REFERENCES

- Adeniji, A. A., and M. Lawal. 2012. Effects of replacing groundnut cake with *Moringa oleifera* leaf meal in the diets of grower rabbits. Int. J. Mol. Vet. Res. 2:8–13.
- Abdulsalam, S., M. Yahaya, and M. Yakasai. 2015. Performance of broiler chickens fed on *Moringa oleifera* leaf meal supplemented poultry feed. Nigeria Agric. J. 46:139–146.
- Ahmad, S., A. Khalique, T. N. Pasha, S. Mehmood, K. Hussain, S. Ahmad, M. S. Shaheen, M. Naeem, and M. Shafiq. 2017. Effect of *Moringa oleifera* (Lam.) pods as feed additive on egg antioxidants, chemical composition and performance of commercial layers. South Afr. J. Anim. Sci. 47:864–874.

- Alabi, O., A. Malik, J. Ng'ambi, P. Obaje, and B. Ojo. 2017. Effect of aqueous *Moringa Oleifera* (Lam) leaf extracts on growth performance and carcass characteristics of Hubbard broiler chicken. Braz. J. Poult. Sci. 19:273–280.
- Alagawany, M., and M. E. Abd El-Hack. 2015. The effect of rosemary herb as a dietary supplement on performance, egg quality, serum biochemical parameters, and oxidative status in laying hens. J. Anim. Feed Sci. 24:341–347.
- Alnidawi, A., F. Ali, S. Abdelgayed, F. Ahmed, and M. Farid. 2016. *Moringa oleifera* leaves in broiler diets: effect on chicken performance and health. Food Sci. Qual. Manag. 58:40–48.
- Babiker, E. E., F. A. L. Juhaimi, K. Ghafoor, and K. A. Abdoun. 2017. Comparative study on feeding value of *Moringa* leaves as a partial replacement for alfalfa hay in ewes and goats. Livest. Sci. 195:21–26.
- Ebenebe, C. I., C. C. Anigbogu, M. A. Anizoba, and A. N. Ufele. 2013. Effect of various levels of *Moringa leaf* meal on egg quality of Isa Brown Breed of layers. Adv. Life Sci. Technology 14:45–49.
- Falowo, A. B., P. O. Fayemi, and V. Muchenje. 2014. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: a review. Food Res. Intern. 64:171–181.
- Gadzirayi, C. T., B. Masamha, J. F. Mupangwa, and S. Washaya. 2012. Performance of broiler chickens fed on mature *moringa oleifera* leaf meal as a protein supplement to soybean meal. Int. J. Poult. Sci. 11:5–10.
- Gakuya, D. W., P. N. Mbugua, B. Kavoi, and S. G. Kiama. 2014. Effect of supplementation of *Moringa oleifera* leaf meal in broiler chicken Feed. Int. J. Poul. Sci. 13:208–213.
- Gupta, A., M. K. Gautam, R. K. Singh, M. V. Kumar, C. V. Rao, R. K. Goel, and S. Anupurba. 2010. Immunomodulatory effect of *Moringa oleifera* Lam. extract on cyclophosphamide induced toxicity in mice. Indian J. Exp. Biol. 48:1157–1160.
- Hammershøj, M., and S. Steenfeldt. 2012. The effects of kale (Brassica oleracea ssp acephala), basil (Ocimum basilicum) and thyme (Thymus vulgaris) as foragematerial in organic egg production on egg quality. Br. Poult. Sci. 53:245–256.
- He, J., G. D. Feng, X. Ao, Y. F. Li, H. X. Qian, J. B. Liu, G. Y. Bai, and Z. Z. He. 2016. Effects of L-glutamine on growth performance, antioxidant ability, immunity and expression of genes related to intestinal health in weanling pigs. Livest. Sci. 189:102–109.
- Hosseinzadeh, H., A. Ahmad, A. Qotbi, A. Seidavi, D. Norris, and D. Brown. 2014. Effects of different levels of coriander (*Coriandrum sativum*) seed powder and extract on serum biochemical parameters, Microbiota, and immunity in broiler Chicks. ScientificWorld J. 24:1–10.
- Lu, W., J. Wang, H. J. Zhang, S. G. Wu, and G. H. Qi. 2016. Evaluation of *Moringa oleifera* leaf in laying hens: effects on laying performance, egg quality, plasma biochemistry and organ histopathological indices. Ital. J. Anim. Sci. 15:658–665.
- Mahfuz, S., and X. S. Piao. 2019. Application of moringa (moringa oleifera) as natural feed supplement in poultry diets. Animals 9:431.
- Makanjuola, B. A., O. O. Obi, T. O. Olorungbohunmi, O. A. Morakinyo, M. O. Oladele-Bukola, and B. A. Boladuro. 2014.

Effect of *Moringa oleifera* leaf meal as a substitute for antibiotics on the performance and blood parameters of broiler chickens. Livestock Res. Rural Development 26:144.

- Mendieta-Araica, B., R. Sporndly, N. Reyes-Sanchez, and E. Sporndly. 2011. Moringa (*Moringa oleifera*) leaf meal as a source of protein in locally produced concentrates for dairy cows fed low protein diets in tropical areas. Livestock Sci. 137:10–17.
- Moreki, J. C., and K. Gabanakgosi. 2014. Potential use of *Moringa* olifera in poultry diets. Glob. J. Anim. Sci. Resh. 2:109–115.
- Moyo, B., P. J. Masika, and V. Muchenje. 2016. Potential use of Moringa oleifera leaf in animal feeding: a Review. Int. J. Curr. Agric. Res. 4:9187–9194.
- Mukumbo, F. E., V. Maphosa, A. Hugo, T. T. Nkukwana, T. P. Mabusela, and V. Muchenje. 2014. Effect of *Moringa oleifera* leaf meal on finisher pig growth performance, meat quality, shelf life and fatty acid composition of pork. South Afr. J. Anim. Sci. 44:388–400.
- Onunkwo, D. N., and O. S. George. 2015. Effects of *Moringa oleifera* leaf meal on the growth performance and carcass characteristics of broiler birds. IOSR J. Agric. Vet. Sci. 8:63–66.
- Rachmawati, I., and M. Rifa'i. 2014. In vitro immunomodulatory activity of aqueous extract of *Moringa oleifera* Lam. leaf to the CD4+, CD8+ and B220+ cells in Mus musculus. J. Exp. Life Sci. 4:15–20.
- Shih, M. C., C. M. Chang, S. M. Kang, and M. L. Tsai. 2011. Effect of different parts (leaf, stem and stalk) and seasons (summer and winter) on the chemical compositions and antioxidant activity of *Moringa Oleifera*. Int. J. Mol. Sci. 12:6077–6088.
- Shah, S. K., D. N. Jhade, and R. Chouksey. 2016. Moringa oleifera Lam. a study of ethnobotany, nutrients and pharmacological profile. Res. J. Pharm. Biol. Chem. Sci. 7:2158–2165.
- Stevens, C. G., F. D. Ugese, G. T. Otitoju, and K. P. Baiyeri. 2015. Proximate and antinutritional composition of leaves and seeds of *Moringa oleifera* in Nigeria: a comparative study. Agro-Science 14:91–97.
- Sudha, P., S. M. Asdaq, S. S. Dhamingi, and G. K. Chandrakala. 2010. Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in animals. Ind. J. Physiol. Pharmacol. 54:133–140.
- Valdez-Solana, M. A., V. Y. Mejía-García, A. Téllez-Valencia, G. García-Arenas, J. Salas-Pacheco, J. J. Alba-Romero, and E. Sierra-Campos. 2015. Nutritional content and elemental and phytochemical analyses of *Moringa oleifera* grown in Mexico. J. Chem. 2015:9.
- Yan, H. L., S. C. Cao, Y. Li, H. F. Zhang, and J. B. Liu. 2019. Reduced meal frequency alleviates high-fat diet-induced lipid accumulation and inflammation in adipose tissue of pigs under the circumstance of fixed feed allowance. Eur. J. Nutr. 59:595–608.
- Yang, L., L. Gang, X. Liang, M. M. Wang, X. ,Q. Zhu, Y. Luo, Y. X. Shang, J. Q. Yang, P. Zhou, and X. L. Gu. 2019. Effects of berberine on the growth performance, antioxidative capacity and immune response to lipopolysaccharide challenge in broilers. Anim. Sci. J. 90:1229–1238.