Effect of dietary inclusion of *Moringa oleifera* leaf on productive performance, egg quality, antioxidant capacity and lipid levels in laying chickens

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**ABSTRACT**

This study was performed to evaluate dietary *Moringa oleifera* leaf powder (MOLP) inclusion on productive performance, egg quality, internal organ index, antioxidant capacity and lipid metabolism in hens at late laying stage. The control (CON) group received basal diet, while the MOLP2.5, MOLP5, MOLP7.5, and MOLP10 groups received basal diet MOLP inclusion at 2.5, 5, 7.5 and 10%, respectively. After six weeks, laying performance, feed intake, egg quality, organ index, antioxidant capacity and lipid indices were analysed. Results showed that low-level dietary of inclusion MOLP (<5%) did not adversely affect laying rate. Feed intake and feed conversion were significantly reduced \((p < 0.05)\) in the MOLP2.5 group. Yolk colour showed linear and quadratic responses to MOLP supplementation and changes in yolk colour stabilised over time. The abdominal fat index decreased linearly as the level of MOLP supplementation increased \((p < 0.05)\). Glucose showed a significant decrease in the MOLP groups, compared with the CON group \((p < 0.05)\). Serum triglycerides and high-density lipoprotein were lowest in the MOLP2.5 group. Serum superoxide dismutase (SOD) levels were significantly higher in the MOLP2.5 group \((p < 0.05)\) and expression levels of *SOD1* and *SOD2* mRNAs were significantly higher in the MOLP2.5 and MOLP7.5 groups than in the CON group \((p < 0.05)\). These results suggest that dietary addition of low-level MOLP improves yolk colour and antioxidant capacity, and reduces feed conversion and abdominal fat index in layer chickens.

**HIGHLIGHTS**

1. Feed intake, feed conversion, abdominal fat index and serum glucose were significantly reduced in group treated with 2.5% moringa oleifera leaf powder.
2. Layer chickens fed dietary moringa oleifera leaf powder inclusion significantly improved yolk color.
3. The antioxidant capacity and lipid performance in group with 2.5% moringa oleifera leaf powder addition was best among the treatment groups.

**Introduction**

Plant species have proven to have important nutritional value for poultry. As a rich source of protein (~21% protein based on dry weight) and essential amino acids (Anwar et al. 2007; Falowo et al. 2018), *Moringa oleifera* Lam (MO) can be used as a source of plant protein for livestock. Nuhu (2010) reported that MO leaf (MOL) meal could be used as a partial substitute for soybean meal in rabbits. A study from Selim et al. (2021) showed that the relative content of the meat \(-3\) polyunsaturated fatty acid (PUFA) content was increased at the 1.5 g/kg MOL level in growing rabbit, because MOL is rich in PUFA (Teixeira et al. 2014). Kholif et al. (2018) found that the dietary inclusion of 375 g/kg MO dry matte in the diets of lactating goats improved feed utilisation and ruminal fermentation, together with milk yield and quality.

Balancing antioxidant status and improving lipid profiles are likely the most studied properties of MO (Fakurazi et al. 2008; Sun et al. 2018; Mabrouki et al. 2020). Extensive research has also shown that MO can be successfully used in poultry feed (Mahfuz and Piao 2019). Cui et al. (2018) showed that when dietary supplementation with MOL was increased from 1 to 10%,
the PUFA content and oxidative stability in breast muscle of AA broilers increased. MOL supplementation in broilers can enhance growth and antioxidant status (Abu Hafsa et al. 2020) and was shown to reduce triglyceride (TG) and total cholesterol (CHO) levels under heat stress (El-Deep et al. 2019). Dietary supplementation with *Moringa setenopetela*, one of the 14 known species of *Moringa*, improved carcase traits in Koekoek chickens (Melesse et al. 2013). Supplementation of broiler feed altered intestinal microarchitecture and acidic mucin production, and had a slight immunomodulatory effect (Khan et al. 2017). As well as providing advantages in broiler chickens, MO can improve egg quality and antioxidant status in commercial layers (Lu et al. 2016), ducks (Yang et al. 2020), and quail (Ashour et al. 2020). Lu et al. (2016) observed that MOL supplementation in Hy-Line Grey commercial layers significantly improved yolk colour and increased serum glutathione peroxidase (GPx) activity, while Yang et al. (2020) found that MO meal positively affected laying performance and reduced serum malondialdehyde (MDA) levels.

Although there are numerous publications describing the effect of dietary inclusion of MOL on chickens, to date there have been few studies carried out in local layers. China local chickens have been developed widely over the past 20 years to improve product quality but there have been no reports of MOL as a feed ingredient in China local chickens, especially in the late laying period. The objective of the present study was to establish the optimum level of MOL in layer diets and to determine its effect on productive performance, egg quality, internal organ indices and antioxidant status during the late laying period.

**Materials and methods**

**Animals and experimental design**

Thirty-seven-week old F1 generation chickens generated from Wenchang chickens and Rugao Yellow chickens were used in the trial, which was carried out in the Poultry Institute, Chinese Academy of Agricultural Sciences. The birds were divided into five groups, CON, MOLP2.5, MOLPS, MOLP7.5 and MOLP10, which received feed with 0, 2.5, 5, 7.5 and 10% MOLP, respectively. Each group had 70 birds (5 replicates, 14 birds in each replicate) and the MO leaf was bought from Yunnan Dayaoshan Trading Co., Ltd (Kunming, Yunnan, China). The main nutritional components were measured according to Official Methods of Analysis (AOAC 2012) before the feed formula was designed (Table 1). The nutritional requirements of the diets and the amount of mineral premix were designed according to the NRC standard (NRC 1994). Ingredients and nutrient levels of the experimental diets are shown in Table 2. The experiment lasted for six weeks after an initial one-week adaptation to dietary MOLP. The chickens were housed in single cages and were given a 16 h light: 8 h dark cycle. Room temperature was maintained at 15 ± 2 °C. Water and food were available *ad libitum* throughout the trial.

| Item | Metabolic energy, MJ/kg | Crude protein, % | Crude fibre, % | Ether extract, % | Available Phosphorus, % | Methionine, % | Lysine, % | Crude Fibre, % |
|------|-------------------------|------------------|----------------|-----------------|------------------------|--------------|----------|-------------|
|      | 7.96                    | 27.60            | 19.26          | 5.90            | 0.320                  | 0.370        | 0.785    | 2.418       |
| %    |                         |                  |                |                 |                        |              |          |             |

Table 2. Ingredients and nutrient levels of experiment diet.

Table 1. Chemical composition of *Moringa oleifera* leaf powder (MOLP) sample on dry matter basis.
**Productive performance**

Hen-day egg production and hen mortality were recorded daily. Egg production is expressed as average hen-day production, calculated from total eggs divided by 42 days (%). Feed intake (g/hen per day) and average egg weight (g) were recorded on a replicate basis at weekly intervals. Feed conversion rate (g feed/g egg) was calculated as the ratio between egg mass and feed consumption on a replicate basis.

**Egg quality**

Thirty eggs per group (six eggs per replicate) were collected at weeks 2, 4, and 6 to measure egg quality parameters, including exterior and interior quality. Egg shape index was calculated as the ratio of the long and short diameters, which were measured using a Vernier calliper. Eggshell colour was measured using a hand-held CM-2600d spectrophotometer (Konica Minolta, Inc., Tokyo, Japan), based on CIE L*a*b* colour space parameters (Samiullah et al. 2015). Eggshell strength was measured using an Egg Force Reader-01 (ORKA Food Technology Ltd., Ramat HaSharon, Israel). Egg weight, albumen height, Haugh unit and yolk colour were measured using an Egg Analyser (ORKA Food Technology Ltd.). The percentage yolk weight of the egg was calculated after separating and weighing the yolk. Finally, the eggshell membrane was cleaned and the eggshell thickness was measured using a micrometer accurate to 0.01 mm and the eggshell was weighed using an electronic balance.

**Calculation of internal organ index**

At the end of the feeding period, two birds were randomly selected from each replicate and denied feed for 12 h. The birds were then weighed and euthanized using carbon dioxide. Internal organs, including heart, liver, spleen, lung, kidney and abdominal fat, were weighed separately and internal organ index were calculated using the following formula: organ index (%) = organ weight/body weight × 100%.

**Antioxidant indices and measurement of lipid parameters**

Superoxide dismutase (SOD), malondialdehyde (MDA) and total antioxidant capacity (T-AOC) indices in serum and liver homogenates were measured using ELISA kits (Jiancheng Company, Jiancheng, Nanjing, China), according to the manufacturer’s instructions. Lipid indicators, including glucose (Glu), triglycerides (TG), total cholesterol (CHO), high-density lipoprotein (HDLC) and low-density lipoprotein (LDLC) in serum and liver homogenates were also determined using ELISA kits (Jiancheng Company), according to the manufacturer’s instructions. All samples were measured in triplicate, at appropriate dilutions. The values of each parameter are expressed according to the test kit.

**RNA extraction and RT-qPCR**

RNA was extracted from liver samples (100 mg) using Trizol reagent (Takara, Dalian, China) and the concentration and quality of RNA were measured using a spectrophotometer (Eppendorf Biotechnology, Hamburg, Germany). Samples with an absorption between 1.8 and 2.1 at 260/280 nm were selected, and six specimens from each group were used for subsequent analysis. Total RNA (1 ug in a final reaction volume of 20 uL) was reverse transcribed into cDNA using a Reverse Transcription Kit (Takara), and the cDNA was analysed by quantitative real-time PCR (RT-qPCR) using SYBR Premix Ex Taq (Takara). The total volume of the RT-qPCR reaction mixture was 20 μL and contained 1 μL cDNA, 10 μL SYBR Premix EX Taq, 1 μL each of primers (10 μM), and 7 μL ddH2O. The reaction conditions were as follows: 95°C for 5 min, 95°C for 30 sec, 60°C for 30 sec and 72°C for 1 min, for 40 cycles; followed by extension at 72°C for 5 min. Primers for the genes catalase (CAT), nuclear factor erythroid 2-related factor 2 (Nrf2), SOD1 and SOD2 are listed in Table 3. All primers were designed and validated by the authors. Gene β-actin mRNA was used as a reference gene for normalisation purposes. Samples were run in triplicate and the 2−ΔΔCT method was used to calculate relative mRNA expression levels.

**Statistical analysis**

All values are represented as the arithmetic mean and standard error of the mean (SEM), with statistical significance determined by One-way ANOVA in SPSS (SPSS 22.0 for Windows, SPSS Inc., Chicago, IL, USA) followed by LSD multiple comparisons. The effects dietary inclusion of MOLP at various levels were evaluated using an orthogonal polynomial contrast test for linear and quadratic effects. p < .05 were considered to be significant differences. Plots were drawn using Prism software (GraphPad Software, Inc., La Jolla, CA, USA).
Results

Chemical composition in MOLP

Table 1 shows the chemical composition of MOLP, which was (on a dry matter basis): 7.96 MJ/kg metabolizable energy, 27.6% crude protein, 19.26% crude fibre, 5.9% ether extract, 6.19% crude ash, 2.2% calcium content, 0.4% phosphorus contents, lysine content 1.83%, and methionine content 0.25%.

Productive performance

The effects of MOLP on productive performance are shown in Table 4. No mortalities were recorded during experimental period. The laying rates were 78.91, 76.90, 74.51, 73.95 and 73.42% in the five groups. The higher level of dietary MOLP inclusion, the lower the laying rate, with the laying rates in the MOLP5, MOLP7.5 and MOLP10 groups significantly lower than in the CON group (p < .05). Notably, the changes of laying rate were significantly higher in MOLP5, MOLP7.5 and MOLP10 groups compared to MOLP2.5 and CON groups (p < .05). Average daily feed intake was 91.22, 83.95, 79.94, 73.87 and 76.20 g, and feed conversion (g of feed/g of egg) was 2.51, 2.47, 2.43, 2.20 and 2.29, respectively, in the five groups. Compared with the CON group, the MOLP groups had significantly lower feed intake and feed conversion (p < .05).

Egg quality

There was no significant difference in egg weight, albumen height, eggshell weight or yolk rate between the groups (Table 5). There was, however, a significant different in eggshell colour L* values in the different groups at the three-week time point, with overall highest L* values in the MOLP10 group (p < .05). Values of a* were significantly different at weeks 2 and 6 (p < .05), whereas values of b* were significantly different at week 4 (p < .05). The egg shape index was reduced by dietary MOLP inclusion, but the differences between the groups were not significant at week 6. Eggshell strength was higher in treatment groups than in the CON group at week 2 and it was higher in the MOLP2.5, MOLP5 and MOLP7.5 groups compared with the CON and MOLP10 groups, with the highest strength in the MOLP7.5 group at week 6. No significant differences in Haugh unit were seen, except for higher value in MOLP5 than the other groups. Notably, yolk colour increased as more MOLP was added to the feed. Yolk colour in the MOLP groups was significantly higher than in the CON group (p < .05) and gradually stabilised over time. Linear and quadratic effects were both observed in yolk colour responses to dietary MOLP at all weeks.

Organ index

Data of internal organ index are shown in Table 6. No significant differences were observed in heart index,
Glucose and lipid metabolism

Analysis of glucose and lipid metabolism

Glucose and lipid metabolism in serum and liver are listed in Table 7. Glucose levels decreased significantly in the treatment groups compared with the CON group (p < .05). No significant differences were observed in serum levels of CHO and LDL-C, nor in any liver indicators. TG levels in serum were significantly lower in the MOLP2.5 and MOLP10 groups than in the CON group (p < .05), whereas HDLC levels in serum were significantly lower in the MOLP2.5 and MOLP7.5 groups than in the CON group (p < .05).

Antioxidant activity and analysis of antioxidant gene-related mRNA

Antioxidant activity indicators are listed in Table 8. No difference in MDA levels in serum or T-AOC levels in liver were observed between the groups.
Serum SOD levels in the MOLP2.5 group were significantly higher than in the other groups \((p < .05)\). MDA activity in the liver was lower in the MOLP5 and MOLP7.5 groups than in the CON group \((p < .05)\). Antioxidant gene-related mRNA expression is shown in Figure 1. There was no significant change in expression of CAT mRNA, whereas expression of Nrf2-related mRNAs was significantly increased in the MOLP10 group, compared with the other groups \((p < .05)\). Expression of SOD1 and SOD2 mRNAs increased with MOLP supplementation; levels in the MOLP2.5 and MOLP7.5 groups were significantly higher than in the CON group \((p < .05)\).
Egg quality

Eggshell colour affects consumer choice. The L* and a* values of overall eggshell colour increased and decreased, respectively, in response to dietary MOLP supplementation. Eggshell colour is affected by the protoporphyrin IX synthetic pathway and some minerals, such as Fe, Cu, Mn and Zn, function as chelating carriers at the central position of the porphyrin molecule (Samiullah et al. 2015). Since MO is rich in mineral elements (Thurber and Fahey 2009; Leone et al. 2015), we speculate that the mineral elements in MOLP impact the synthesis or transport of protoporphyrin.

It is worth noting that, with increasing MOLP supplementation, the yolk colour increased significantly but tended to stabilise over time. N‘Nanle et al. (2020) and Lu et al. (2016) similarly found that MOL has a marked effect on yolk colour, which is mainly affected by lipid-soluble pigments that are synthesised from α-carotene, β-carotene, lutein and carotenoids. MOLP, which is rich in vitamins (Leone et al. 2015), may facilitate the synthesis of lipid-soluble pigments. In markets where eggs with high yolk colour are valued, MOLP supplementation in layers would raise egg prices and increase economic efficiency.

Organ index and lipid metabolism

Organ index, an important fitness trait, reflect the health status of an animal and are considered to be relevant to modern broiler chickens (Deeb and Lamont 2002). We found that most organ indices, except the lung index and abdominal fat index, were unchanged by increasing MOLP supplementation, suggesting that MOLP has no adverse effects on fitness traits. Notably, the increased lung index may lead to increased activity time and energy intake, which will be beneficial to animal welfare level. It has been shown that excessive deposition of abdominal fat has a negative influence on ovarian hierarchy and the laying performance of chickens (McDerment et al. 2012). The lower laying rate of Chinese local chickens in the late laying period could be due to greater fat deposition compared with highly efficient commercial hens (our unpublished data). Here, we found that the abdominal fat index decreased with increasing MOLP supplementation. Previous studies generally also found that dietary supplementation with natural plant materials could suppress the deposition of abdominal fat in laying hens (Zou and Lu 2002). Abdominal fat is affected by serum biochemical indices (Musa et al. 2014). In this study, we found that serum TG, CHO,
HDLC and LDLC levels decreased in response to dietary MOLP inclusion, and that TG and HDLC levels were significantly reduced in the MOLP2.5 group \((p < .05)\). These results are in line with those of N’Nanle et al. (2020) suggesting that similar mechanisms may underlie the regulation of serum lipid metabolism by MO in mice and humans (Ghasi et al. 2000; Chumark et al. 2008).

MOLP reduced liver lipid levels in some treatment groups, but the differences were not dose dependent. Changes in liver lipids were not as extensive as those in serum lipids, suggesting that lipid synthesis, metabolism and transportation had changed, but not the structure of the liver. This interpretation is in agreement with a study by Lu et al. (2016) which showed that 15% dietary MOLP changed the histopathology of the liver.

We also found that dietary MOLP inclusion significantly decreased serum glucose concentrations, which is similar to the results of Chen et al. (2020) and suggests that MOLP increases oxidative glucose metabolism. MOL has been reported to have antihyperglycemic effects in both mouse and human studies (Stohs and Hartman 2015; Amelia et al. 2018). Accordingly, MOLP supplementation potentially has a glucose- and lipid-lowering effect in laying chickens, thus promoting animal welfare. In order to distinguish more precisely between the effects of MOL on fat storage and lipid metabolism, future MOLP studies should measure lipid metabolism-related genes or pathways.

**Antioxidant activity**

SOD and T-AOC are the main antioxidant enzymes in the liver and play a key role in balancing oxidants and antioxidants. Previous studies have demonstrated that phytogenic feed additives may increase serum antioxidant capacity. We found that dietary MOLP inclusion significantly increased serum SOD and T-AOC levels in the MOLP2.5 group. This result is slightly different from that obtained by Cui et al. (2018) in broiler chickens since their study showed that serum SOD and T-AOC levels increased quadratically. The increased serum levels of SOD and T-AOC may be attributed to the increased flavonoids and vitamin C in MOLP. MDA can be used to assess oxidative damage in lipids. We saw no changes in serum MDA levels in this study, but liver MDA levels were significantly lower in the MOLP5 group than in the other groups. This is different from the findings of Cui et al. (2018) in broiler chickens and of Lu et al. (2016) in commercial laying chickens. Differences in the effects of MOLP on chickens are likely to be attributable to differences in breed and feeding stage.

Previous studies using phytogenic extracts showed that activation of the Nrf2 pathway promotes antioxidant activity in broiler chickens (Shen et al. 2019). In line with previous findings, we saw an increase in Nrf2 mRNA in the liver in the MOLP10 group, indicating that higher levels of MOLP supplementation stimulate antioxidant activity in Chinese local laying chickens. Expression of SOD2 mRNA showed significant favourable changes under MOLP supplementation and expression of SOD1 mRNA was significantly higher in the MOLP2.5 and MOLP7.5 groups, compared with the CON group \((p < .05)\). The increased levels of SOD, T-AOC activity and expression of Nrf2 and SOD mRNA demonstrate the potential of MOL to regulate antioxidant activity in laying hens. Our comprehensive analysis showed that supplementation with 2.5% MOLP provided the greatest effect on antioxidant capacity.

**Conclusion**

This study provides further evidence that dietary inclusion of 2.5% MOLP in hens does not negatively affect laying performance and is good for yolk colour. Dietary MOLP inclusion can regulate oxidation and lipid metabolism in China local chickens. Although Nrf2 and SOD genes were found to have antioxidant properties, the underlying mechanisms need further investigation in future studies. Our study confirms that natural plant extracts, such as MOLP, potentially have a lipid-lowering effect.

**Ethical approval**

Use of animals and sample collection followed the guidelines established by the Ministry of Agriculture of China. All procedures were approved by the Institution Animal Care and Use Committee of Jiangsu University of Science and Technology.

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**Disclosure statement**

We certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript. No potential conflict of interest was reported by the author(s).
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