https://doi.org/10.17221/166/2021-CJAS

Effect of dietary supplementation with mulberry and moringa leaves on the chicken reproductive performance

Manman Shen[1,2#], Tao Li[1#], Jian Lu[2], Kehua Wang[2], Liang Qu[2], Qirui Hou[1], Weiguo Zhao[1], Ping Wu[1]*

[1]*Jiangsu Key Laboratory of Sericultural Biology and Biotechnology, School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang, P.R. China*
[2]*Jiangsu Institute of Poultry Science, Yangzhou, P.R. China*

[*]*Corresponding authors: wgzsri@126.com*
[#]*These authors contributed equally to this work*

Citation: Shen M.M., Li T., Lu J., Wang K.H., Qu L., Hou Q.R., Zhao W.G., Wu P. (2022): Effect of dietary supplementation with mulberry and moringa leaves on chicken reproductive performance. Czech J. Anim. Sci., 67: 339–347.

Abstract: The effects of dietary supplementation with moringa *(Moringa oleifera* Lam.) and mulberry (*Morus nigra* L.) leaf powder on the clutch trait, reproductive organs, follicles, steroid hormones, and gene expressions of laying chickens were investigated during a 42-day experiment. Two hundred and ten Chinese local strain hens aged 37 weeks were randomly allocated to three groups, each group included five replicates, with 14 hens per replicate. The hens in the control group were fed with the basal diet, whereas those in the test groups were fed with a basal diet supplemented with 2.5% moringa leaf powder and 2.5% mulberry leaf powder (MOLP2.5+MLP2.5), 5% MOLP and 2.5% MLP (MOLP5+MLP2.5). The clutch traits (clutches, clutch length, delay days) were recorded during the trial. At the end of the experiment, the effect of the dietary supplementation with MOLP and MLP on the reproductive organs and tissues was estimated, and the gene expressions of *ESR1*, *ESR2*, *CYP19A1* and *STAR* were analysed. Compared with the control group, the clutch performed worse in the treatment groups, however, the laying rate, reproductive organs and tissues in the MOLP2.5+MLP2.5 group showed no significant difference. Though the oestrogen levels did not significantly increase, they were elevated in the MOLP2.5+MLP2.5 group. Furthermore, it was identified that the expression levels of the *ESR1* and *CYP19A1* mRNA were significantly upregulated in the MOLP2.5+MLP2.5 group compared with those in the control group. In conclusion, a low addition level of MOLP and MLP can be used in laying hens to improve the steroid hormones synthesis-related gene expression which might improve the reproductive performance over the long term.

Keywords: clutch; layer; follicle; phytogenic feed additive; steroid hormone

Mulberry (*Morus nigra* L.) is classified as a broad-leaved plant, its leaves are rich source of protein (14.0–34.2%), metabolic energy (1 130–2 240 kcal/kg), and high dry matter digestibility (75–85%) (Hassan et al. 2020). Mulberry leaves are not only commonly used by silkworms and irreplaceable in the sericul-

Supported by the Earmarked Fund (No. CARS-18), National Key R&D Program of China (No. 2021YFE0111100), Zhenjiang Science and Technology Support Project (No. GJ2021015), Guangxi Innovation-Driven Development Project (No. AA19182012-2) and China Agriculture Research Systems (No. CARS-40).

https://doi.org/10.17221/166/2021-CJAS

ture industry, but also have a hypoglycaemic effect (Zhang et al. 2019a). Given the rapid development of the intensive silkworm culture increased in the world, a large amount of the mulberry leaves remain unused. Studies have shown that supplementary mulberry leaves in livestock or poultry can improve the animal performance and the quality of the products, thus, it is considered a potential resource for feed (Liu et al. 2021). A dietary supplement complemented by 25.5% fermented mulberry leaves in a gestational sow increased the voluntary feed intake and weaning litter weight (Zhang et al. 2021). A dietary supplementation with 4% mulberry leaf powder greatly affected the gut microbiota of chickens by changing the gut microbiota compositions (Chen et al. 2019). With the rising feed costs caused by the increasing demand for grains and mulberry rich in nutrients, it is imperative to develop mulberry leaves as well as phytogenic feeds for use as animal alternative materials for major crop feedstuffs.

It has been reported that a combined effect can be obtained if different feed supplementations are used together in animal diets. Goncalves et al. (2019) found an interactive relationship between fishmeal and a phytogenic product in marine carnivorous fish species. A study by Miao et al. (2020) showed that mulberry leaves and a bamboo charcoal additive had a combined effect on the growth performance, lipid metabolism, and anti-oxidant in farmed tilapia. A study by Valacchi et al. (2014) showed that the combined mulberry leaves and fruit extract had beneficial effects on the modulation of high-fat diet-induced oxidative stress. Therefore, continued investigations into the combined use of the mulberry and a phytogenic feed are required.

Moringa (*Moringa oleifera* Lam.) is a popular tree across the world and has been used in poultry production (Mahfuz and Piao 2019). Studies have shown that a suitable supplementation level of moringa leaves was 5% in Hy-Line Grey hens (Lu et al. 2016) and 1.56% in Arbor Acres broilers (Cui et al. 2018). The moringa has an important role on the production traits, such as increasing the litter size (Zeng et al. 2019), modifying the pork fatty acid profile (Zhang et al. 2019b) and improving the follicle development (Amelia et al. 2018). In China, moringa is widely grown and its planting area exceeds 2 667 hectares. Although a number of moringa has been used for food product, it has rarely been used for feed. Therefore, it is necessary to apply moringa in animal feeds. However, there is no relevant research on the combination between mulberry leaves and moringa leaves in poultry, especially in terms of the reproductive performance.

The hen reproductive performance is an important factor in the profitability of chicken production. The reproductive performance of hens depends on many factors, such as the breed, age, nutrition, and management. The use of phytogenic substances is increasing in the poultry industry because these materials are confirmed to be safe and effective in improving the laying performance. Based on the considerations mentioned above, this study was conducted to elucidate the combination effect of moringa leaves and mulberry leaves on the reproductive performance in laying hens.

MATERIAL AND METHODS

Moringa and mulberry leaf powder preparation

Fresh moringa leaves were bought from Yunnan Daoshan Co. Ltd (Yunnan, China). Fresh mulberry leaves were collected from the Chinese Academy of Agricultural Sciences. The fresh leaves were air-dried with no direct sun exposure and kept at room temperature. After drying, the leaves were ground into a fine powder. The analysed nutrient structure of the moringa and mulberry leaves were measured according to the Association of Official Analytical Chemists (AOAC) methods (AOAC 2004) for the crude protein (method 988.05), crude fibre (method 978.10), ether extract (method 920.19), crude ash (method 942.05), calcium (method 927.02), phosphorus (method 995.11), lysine (method 975.44), and methionine (method 994.12). The metabolisable energy was calculated according to the United States Department of Agriculture (USDA) database (https://ndb.nal.usda.gov/ndb/), based on the Atwater factor system. The total phenolics and total flavonoids were determined according to Meda et al. (2005). The nutrient level of the moringa leaves and mulberry leaves are listed in Table 1.

Hens and experimental design

The hens were provided by the Jiangsu Institute of Poultry Science. A total of 210 F1 hens aged

https://doi.org/10.17221/166/2021-CJAS

Table 1. Chemical composition of the moringa and mulberry leaves on a dry matter basis

Item (%)	Moringa leaves	Mulberry leaves
Metabolic energy (MJ/kg)	8.51	7.15
Crude protein	27.60	13.79
Crude fiber	19.26	24.89
Ether extract	5.76	1.98
Crude ash	6.19	5.75
Calcium	2.20	4.0
Phosphorus	0.40	0.45
Lysine	1.83	0.65
Methionine	0.25	0.13
Phenolic	44.37 GAE mg/g	30.57 GAE mg/g
Total flavonoids	23.78 QE mg/g	55.42 QE mg/g

GAE = gallic acid equivalents; QE = quercetin equivalents

37 weeks produced from the Wenchang Chicken and the Rugao Yellow Chicken, both of which are Chinese local strain chickens, were randomly distributed into three groups, subdivided into five replicate groups containing 14 hens each. One bird was raised per cage during the whole period of the experiment. The hens were given food and water *ad libitum*, while maintaining a lighting cycle of 16 h light : 8 h dark. The clutch and laying rate were matched in each group, with no significant differences before the beginning of the experiment. The different ratio of the moringa leaf powder (MOLP) and mulberry leaf powder (MLP) treatment lasted for seven weeks, including a 1-week pre-experimental period. The three groups were fed with the basal diet (control group), the basal diet + 2.5% MOLP + 2.5% MLP (MOLP2.5+MLP2.5) and the basal diet + 5% MOLP + 2.5% MLP (MOLP5+MLP2.5). The basal diets were made according to the Management Guide of the National Research Council (NRC 1994). The composition and the nutrient levels of the diets are calculated and listed in Table 2. All the research procedures were approved by the Institutional Animal Care and Use Committee of Jiangsu University of Science and Technology (SYXK[Su]2016-2020).

Clutch assessments

The hen mortality was recorded daily during the experiment. A clutch was considered as the number of eggs laid on consecutive days without missing. Starting with the first egg laid by a hen at the beginning of the experiment, the number of clutches, average number of eggs in a clutch defined as the average clutch length, the maximum number of eggs in a clutch defined as the maximal clutch length, and the clutch intensity obtained from clutch length divided by clutches were measured.

Reproductive organs and tissues collection

At the end of the experiment, two hens/replicate were randomly collected. Blood samples were collected from the wing vein for the separation of the serum (centrifugation at 3 000 rpm for 10 min). Then, the serum was stored at –20 °C until analysis. A total of 30 hens were slaughtered to obtain the ovary and oviduct. The hierarchical follicles (diameter > 10 mm) and small yellow follicles (diameter between 4 mm and 10 mm) were separated and counted. Then, the weight of the hierarchical follicles, ovarian stroma excluding all the hierarchical follicles and small yellow follicles, and oviduct were measured. The length of the oviduct was also measured. The ovarian stroma samples excluding all the follicles with a diameter > 2 mm were taken and immediately frozen with liquid nitrogen, and stored at –80 °C until use.

Steroid detection

The levels of oestrogen and progesterone were estimated using commercial enzyme-linked immunosorbent assay (ELISA) test kits (Jiancheng Co., Ltd, Nanjing, China) following the manufacturer's instructions.

Gene expressions

The total RNA of the ovarian stroma was extracted using a Trizol kit (Takara Co., Ltd, Dalian, China) according to the manufacturer's protocol. The RNA purity and integrity were confirmed by Nano-Drop spectrophotometry (Thermo Scientific, Wilmington, DE, USA) and gel electrophoresis. Samples with the ratio of OD 260/280 that ranged from 1.8 to 2.1 were used in the subsequent experiment. Then, we synthesised the cDNA and performed a quantitative real-time polymerase

https://doi.org/10.17221/166/2021-CJAS

Table 2. Composition of the experimental diets supplemented with different MOLP and MLP ratios fed to chicken

Basal diets	Groups (%)		
	control	2.5% MOLP + 2.5% MLP	5% MOLP + 2.5% MLP
Ingredient (%)			
Corn	64.420	63.092	62.605
Soybean meal	23.520	21.440	19.975
Shell powder	6.700	6.700	6.700
NaCl	0.300	0.300	0.300
Calcium hydrogen phosphate	0.878	0.810	0.780
Limestone	2.107	1.75	1.635
Zeolite powder	1.632	0.459	0.053
50% choline chloride	0.170	0.170	0.170
DL-Methionine	0.116	0.122	0.125
Lysine	0.100	0.100	0.100
Vitamin/trace mineral premix*	0.050	0.050	0.050
Phytase	0.007	0.007	0.007
Nutrient levels[1]			
Metabolizable energy (MJ/kg)	11.087 4	11.087 0	11.087 2
Crude protein (%)	16.000	16.000	16.000
Crude fibre (%)	2.418	3.378	3.765
Lysine (%)	0.785	0.785	0.793
Methionine (%)	0.370	0.370	0.370
Calcium (% DM)	3.35	3.35	3.35
Phosphorus (% DM)	0.32	0.32	0.32

MLP = mulberry leaf powder; MOLP = moringa leaf powder

*Premix provided per kilogram of diet: vitamin A (retinyl palmitate), 7 715 IU; vitamin D_3 (cholecalciferol), 2 755 international chick units; vitamin E (DL-tocopheryl acetate), 8.8 IU; vitamin K (menadione sodium bisulfate complex), 2.2 mg; vitamin B_{12} (cobalamin), 0.01 mg; menadione (menadione sodium bisulfate complex), 0.18 mg; riboflavin, 4.41 mg; pantothenic acid (D-calcium pantothenate), 5.51 mg; niacin, 19.8 mg; folic acid, 0.28 mg; pyridoxine (pyridoxine hydrochloride), 0.55 mg; manganese (manganese sulfate), 50 mg; iron (ferrous sulfate), 25 mg; copper (copper sulfate), 2.5 mg; zinc (zinc sulfate), 50 mg; iodine (calcium iodate), 1.0 mg; selenium (sodium selenite), 0.15 mg

[1]Calculated value

chain reaction (RT-qPCR) analysis using a Reverse Transcription Kit and SYBR Premix Ex Taq (Takara Co., Ltd, Dalian, China). The expression levels of the genes of oestrogen receptor 1 (*ESR1*), oestrogen receptor 2 (*ESR2*), cytochrome P450 family 19 subfamily A member 1 (*CYP19A1*), and steroidogenic acute regulatory protein (*STAR*) were detected. The gene *ACTB* was used as a reference gene for normalisation purposes. The primer sequences of *ACTB* were designed in our laboratory. The other primers were designed to amplify an intron-spanning region using Primer 3.0. The primer sequences are listed in Table 3. The amplification conditions were as follows: 95 °C for 5 min, 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, for 40 cycles in total; extension at 72 °C for 5 minutes. Samples were run in triplicates and the $2^{-\Delta\Delta CT}$ values for the relative mRNA expression were calculated.

Statistical analysis

The data were analysed by an analysis of variance (ANOVA) taking the different MOLP and MLP ratios as the independent variables and the clutch, organ index, follicle trait, hormone level and gene expression level as the dependent variables. The level of significance was indicated by a probability of equal to or less than 0.05. The data were further subjected to multi-variable regression to analyse the different effects of the MOLP and MLP ratios on all the measured variables.

https://doi.org/10.17221/166/2021-CJAS

Table 3. Primer sequences used in the current study

Gene	Primer sequence (5'–3')	Product size (bp)	GeneBank accession No.
ACTB	F: CAGCCATCTTTCTTGGGTAT R: CTGTGATCTCCTTCTGCATCC	169	NM_205518.1
ESR1	F: ACCAACCTTGCAGACAGAGA R: CTAACCAGGCACATTCCAGC	115	NM_205183.2
ESR2	F: ACATCTGCCCAGCTACCAAT R: TCTTTTACACGGGTTGCAGC	214	NM_204794.3
CYP19A1	F: GGCCTCTCATTTCACATGGG R: GCTTGCTCCCAAATCGAGAA	189	NM_001364699.2
STAR	F: GGTGGACAACATGGAGCAGA R: GAGCACCGAACACTCACAAA	159	NM_204686.3

ACTB = actin β; *CYP19A1* = cytochrome P450 family 19 subfamily A member 1; *ESR1* = oestrogen receptor 1; *ESR2* = oestrogen receptor 2; *STAR* = steroidogenic acute regulatory protein

RESULTS

Clutch performance of laying hens

The effects of the dietary supplementation of MOLP and MLP in different ratios on the clutch traits are summarised in Table 4. The number of clutches significantly increased (P = 0.04), whereas the clutch length and maximum clutch length showed a significantly decreasing trend (P < 0.05) in the treatment groups compared to that in the control group. In turn, increasing the concentration of MOLP to 5% of the diet resulted in a significantly lower clutch intensity in relation to the MOLP 2.5% group (P = 0.04).

Reproductive organs and tissues

The data regarding the MOLP and MLP impact on the reproductive organs and tissues are shown in Table 5. No differences were observed in any of the traits (P > 0.05).

Oestrogen and progesterone level

As shown in Table 6, the oestrogen levels in the serum showed an increase in the MOLP2.5+MLP2.5 group, but it was not significant (P > 0.05). The progesterone level decreased with the increasing MOLP supplementation level, but was not significant (P > 0.05)

Gene expressions

The mRNA expression levels of the reproductive genes are presented in Figure 1. The expression levels of *ESR1* and *CYP19A1* were significantly upregulated in the MOLP2.5+MLP2.5 group compared with those in the control group (P < 0.05).

Table 4. Effects of the dietary treatments on the clutch traits in the laying chickens

Variables	Groups			SEM	*P*-value
	control	2.5% MOLP + 2.5% MLP	5% MOLP + 2.5% MLP		
Laying rate (%)	78.91^{a}	76.94^{a}	73.64^{b}	0.618	0.002
Number of clutches	7.51^{b}	8.48^{a}	8.53^{a}	0.18	0.04
Average clutch length (days)	4.77^{a}	3.95^{b}	3.70^{b}	0.12	0.001
Maximal clutch length (days)	9.77^{a}	8.26^{b}	7.19^{c}	0.27	0.000
Delay days (days)	1.28	1.12	1.14	0.05	0.41
Maximal delay days (days)	1.39^{b}	1.59^{b}	3.18^{a}	0.23	0.002
Clutch intensity	0.75^{a}	0.74^{a}	0.72^{b}	0.01	0.06

MLP = mulberry leaf powder; MOLP = moringa leaf powder; SEM = standard error of means
$^{a–c}$Values with different characters in superscripts were different (P < 0.05) in the same row

https://doi.org/10.17221/166/2021-CJAS

Table 5. Effects of the dietary treatments on the reproductive organs and tissues in the laying chickens

Variables	Groups			SEM	*P*-value
	control	2.5% MOLP + 2.5% MLP	5% MOLP + 2.5% MLP		
Ovary weight (g)	4.25	4.05	4.53	1.099	0.66
Hierarchal follicle number	3.50	3.40	3.80	1.006	0.67
Hierarchal follicle weight (g)	21.34	21.58	23.91	0.183	0.64
Small yellow follicle number	9.40	9.13	8.60	0.744	0.91
Oviduct length (cm)	43.80	45.20	41.56	0.999	0.35
Oviduct weight (g)	38.64	40.37	42.07	1.170	0.51

MLP = mulberry leaf powder; MOLP = moringa leaf powder; SEM = standard error of means

Table 6. Effects of the different MOLP and MLP ratios on the oestrogen and progesterone levels in the serum of the layer chickens

Variables (ng/l)	Groups			SEM	*P*-value
	control	2.5% MOLP + 2.5% MLP	2.5% MOLP + 5% MLP		
Estrogen	169.83	177.97	148.10	5.83	0.47
Progesterone	6.44	5.42	4.84	0.77	0.57

MLP = mulberry leaf powder; MOLP = moringa leaf powder; SEM = standard error of means

ESR2 and *STAR* in the treatment groups showed no significant difference when compared with the control group ($P > 0.05$).

DISCUSSION

Generally, a better performance, including the laying rate, feed conversion, and egg quality, could be obtained for a suitable amount of mulberry or moringa added into the poultry feed. A previous study showed that an MLP supplementation level under 2% had no significant effect on the laying rate (Lin et al. 2017). Our team showed that the optimal supplementation level of the mulberry leaf powder (MLP) should be less than 4% in the basal diet in Blue eggshell chickens (Wu et al. 2014). Considering the chickens used in the current experiment were Chinese local chickens, we designed the supplemented level of MLP as 2.5%. In terms of the moringa, it has been suggested that a level of moringa extract at 1% added in brown laying hens and Sasso breeder hens was safe for laying performance (Chen et al. 2020; N'nanle et al. 2020). The supplementation level of moringa stem meal at 4% significantly increased the laying rate,

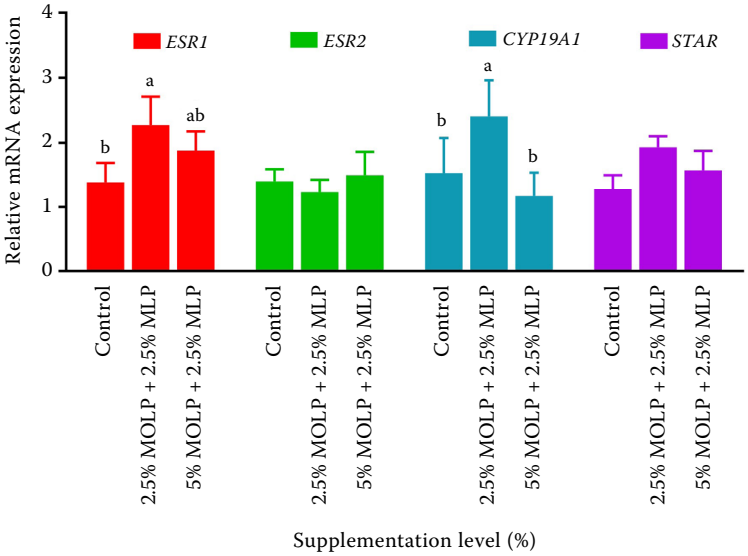

Figure 1. Relative expression of the reproductive genes in the ovaries of the laying chickens upon the MOLP and MLP treatment
CYP19A1 = cytochrome P450 family 19 subfamily A member 1; *ESR1* = oestrogen receptor 1; *ESR2* = oestrogen receptor 2; MLP = mulberry leaf powder; MOLP = moringa leaf powder; *STAR* = steroidogenic acute regulatory protein
[a,b]Bars with different letters are significantly different at $P < 0.05$

https://doi.org/10.17221/166/2021-CJAS

egg weight, and daily feed intake in laying ducks (Yang et al. 2020). However, a higher additional level of MOLP had a side effect on the performance, especially the laying rate (Lu et al. 2016). Therefore, the final highest total supplementation level of MOLP and MLP was decided to be 7.5% in the basal diet, at a fixed 2.5% MLP.

Until now, no studies have reported the effects on the clutch traits with an MLP or MOLP supplementation, let alone with their combination. From the current study, we can draw the conclusion that the MOLP and MLP supplementation showed a negative effect on the clutch traits. The reason may be due to the heterogeneous composition of the used supplements as well as the type of raw materials, including the crude protein, crude fibre content, and concentrations of additives used in the diets. The available literature showed that mulberry leaves are rich in protein (14–34.2%), crude fibre (26.5–36.7%), as well as phytochemical classes, such as flavonoids, benzofurans, phenolic acids, etc. (Chen et al. 2021). Moringa leaves have a similar nutrition level with the protein within a range of 10.74% to 30.29% containing more than 200 compounds (Selim et al. 2021). The crude fibre of the moringa and mulberry was 19.26% and 24.89% in the current study, respectively. The contents were equivalent to 3.378% or 3.765% of crude fibre in the treatment groups. The dietary fibre was considered a diluent of the poultry diet and the optimum supplementation should not exceed 3.88% in hens (Selim and Hussein 2020). Mechanistically, the laying rate and clutch performance decline could occur via modulating the signal transduction mechanism necessary to regulate the metabolism of the absorbed nutrients. However, the contribution of the crude fibre on the laying performance needs further study. The total levels of the total MOLP and MLP supplementation were 5% and 7.5% in our treatment groups, respectively, which were higher than that of the recommended individual MOLP or MLP addition. Thus, it appears that the addition of MOLP or MLP alone or combined together requires a total additional level under 5%.

Moringa and mulberry contain abundant bioactive compounds which hold potent biological activities proven to exhibit excellent pharmacological effects against various diseases (Chen et al. 2021). However, no results about the follicle development upon the MOLP or MLP supplementation have been reported. The present study showed that the MOLP and MLP supplementation together in the laying hens diet had no effect on the reproductive organs and tissues. The level of the oestrogen concentration in the serum was slightly increased in the MOLP2.5+MLP2.5 group, but there was no significance compared with the control group. Only *ESR1* and *CYP19A1* were identified to exhibit marked alterations in the expression levels, no significant difference was found in terms of the hormone and reproductive organs or tissues. STAR is required for progesterone production, with transport of cholesterol into the inner mitochondrial membrane by STAR as the rate-limiting step of progesterone production, plays an important role in the follicular development (Sechman et al. 2020). *CYP19A1* encodes, for the cytochrome P450 aromatase, an enzyme that is responsible for the last step synthesis of the oestrogen. A previous study showed that the higher expression levels of *CYP19A1* were related to the laying performance of ducks (Ren et al. 2019). The phenotype is influenced by long-term gene signalling and hormone secretion, where hormonal secretion fluctuations are in constant flux. The experimental period in the present research might be too short to induce significant changes in the reproduction physiology.

The bioactive compounds include flavonoids and polyphenol, which have important roles in the reproductive performance. There is evidence that flavonoids, also known as phytoestrogens, may modulate the steroid hormone levels through either an estrogenic or antiestrogenic effect as a binding to oestrogen receptors (Moreira et al. 2014). In laying hens, phytoestrogen could affect target tissues by modifying the expression of steroid hormone receptors and then increasing the serum oestrogen (Yang et al. 2018). Regarding the effect of MOLP or MLP on the hormone and gene expression, it was found that the rate of maturation of sheep oocytes was improved with the combined supplementation of a moringa extract and hormones including PMSG, hCG and 17β-oestradiol (Barakat et al. 2015). In mice, a diet with 4% moringa leaves improved the litter size, but the gene expression level of *ESR2* was unaffected (Zeng et al. 2019). Astragalin, a flavonoid from the mulberry, increased the endogenous oestrogen and progesterone at a menopausal age rate model (Wei et al. 2016). It is speculated that the flavonoids from MOLP and MLP may serve as an agonist to combine with oestrogen-binding sites.

https://doi.org/10.17221/166/2021-CJAS

CONCLUSION

Our data revealed that a supplementation level of 2.5% MOLP and 2.5% MLP had no side effect on the laying rate and clutch intensity, but decreased the clutch length. Though the reproductive organs, tissues, and hormone levels showed no significant changes upon the MOLP and MLP supplementation, the steroid biosynthesis-related genes of the *ESR1* and *CYP19A1* mRNA expression levels were increased. The contradictory relationship between the gene expression and the phenotypic appearances needs further study to assess the long-term effects.

Acknowledgment

We thank the colleagues of Jiangsu Institute of Poultry Science for their valuable help.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Amelia D, Santoso B, Purwanto B, Miftahussurur M, Joewono HT. Effects of Moringa oleifera on insulin levels and folliculogenesis in polycystic ovary syndrome model with insulin resistance. Immunol Endocr Metab Agents Med Chem. 2018 May 1;18(1):22-30.

AOAC – Association of Official Analytic Chemists. Official methods of analysis. 15th ed. Washington, DC, USA: Association of Official Analytical Chemists; 2004.

Barakat IA, Khalil WK, Al-Himaidi AR. Moringa oleifera extract modulates the expression of fertility related genes and elevation of calcium ions in sheep oocytes. Small Rumin Res. 2015 Jun 29;130:67-75.

Chen Y, Ni J, Li H. Effect of green tea and mulberry leaf powders on the gut microbiota of chicken. BMC Vet Res. 2019 Mar 6;15(1): 6 p.

Chen ZM, Chang WH, Zheng AJ, Cai HY, Liu GH. Tolerance evaluation of Moringa oleifera extract to Hailan brown laying hens. J Anim Physiol Anim Nutr. 2020 Sep; 104(5):1375-83.

Chen C, Mohamad Razali UH, Saikim FH, Mahyudin A, Mohd Noor NI. Morus alba L. plant: Bioactive compounds and potential as a functional food ingredient. Foods. 2021 Mar 23;10(3): 28 p.

Cui YM, Wang J, Lu W, Zhang HJ, Wu SG, Qi GH. Effect of dietary supplementation with Moringa oleifera leaf on performance, meat quality, and oxidative stability of meat in broilers. Poult Sci. 2018 Aug 1;97(8):2836-44.

Goncalves RA, Serradeiro R, Machado M, Costas B, Hunger C, Dias JAS. Interactive effects of dietary fishmeal level and plant essential oils supplementation on European sea bass, Dicentrarchus labrax: Growth performance, nutrient utilization, and immunological response. World Aquacul Soci. 2019 May 7;50(6):1078-92.

Hassan FU, Arshad MA, Li M, Rehman MS, Loor JJ, Huang J. Potential of mulberry leaf biomass and its flavonoids to improve production and health in ruminants: Mechanistic insights and prospects. Animals. 2020 Nov 9;10(11): 24 p.

Lin WC, Lee MT, Chang SC, Chang YL, Shih CH, Yu B, Lee TT. Effects of mulberry leaves on production performance and the potential modulation of antioxidative status in laying hens. Poult Sci. 2017 May 1;96(5):1191-203.

Liu Y, Li Y, Xiao Y, Peng Y, He J, Chen C, Xiao D, Yin Y, Li F. Mulberry leaf powder regulates antioxidative capacity and lipid metabolism in finishing pigs. Anim Nutr. 2021 Jun 1;7(2):421-9.

Lu W, Wang J, Zhang HJ, Wu SG, Qi GH. Evaluation of Moringa oleifera leaf in laying hens: Effects on laying performance, egg quality, plasma biochemistry and organ histopathological indices. Ital J Anim Sci. 2016 Oct 1;15(4):658-65.

Mahfuz S, Piao XS. Application of moringa (Moringa oleifera) as natural feed supplement in poultry diets. Animals. 2019 Jul 9;9(7): 19 p.

Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 2005 Jul 1;91(3):571-7.

Miao L, Charles O, Lin Y, Gong Y, Zhu W, Wang L, Fu J, Zhang Z, Dong Z. Interactive effects of mulberry leaf meal and bamboo charcoal additive on growth performance, anti-oxidant capacity, and disease resistance of genetically improved farmed tilapia (GIFT) juvenile (Oreochromis niloticus). Aquacult Rep. 2020 Nov 1;18: 8 p.

Moreira AC, Silva AM, Santos MS, Sardao VA. Phytoestrogens as alternative hormone replacement therapy in menopause: What is real, what is unknown. J Steroid Biochem Mol Biol. 2014 Sep 9;143:61-71.

N'nanle O, Tete-Benissan A, Nideou D, Onagbesan OM, Tona K. Use of Moringa oleifera leaves in broiler production chain. 1 – Effect on Sasso breeder hens performances, internal quality of hatching eggs and serum lipids. Vet Med Sci. 2020 Jan 17;6(3):485-90.

NRC – National Research Council. Nutrient requiements of poultry. 9th rev. ed. Washington, DC, USA: National Academies Press; 1994. 176 p.

https://doi.org/10.17221/166/2021-CJAS

Ren J, Sun C, Chen L, Hu J, Huang X, Liu X, Lu L. Exploring differentially expressed key genes related to development of follicle by RNA-seq in Peking ducks (Anas platyrhynchos). PloS One. 2019 Jun 25;14(6): 14 p.

Sechman A, Grzegorzewska AK, Grzesiak M, Kozubek A, Katarzynska-Banasik D, Kowalik K, Hrabia A. Nitrophenols suppress steroidogenesis in prehierarchical chicken ovarian follicles by targeting STAR, HSD3B1, and CYP19A1 and downregulating LH and estrogen receptor expression. Domest Anim Endocrinol. 2020 Jan 1;70: 106378.

Selim S, Hussein E. Production performance, egg quality, blood biochemical constituents, egg yolk lipid profile and lipid peroxidation of laying hens fed sugar beet pulp. Food Chem. 2020 Apr 25;310: 125864.

Selim S, Seleiman MF, Hassan MM, Saleh AA, Mousa MA. Impact of dietary supplementation with Moringa oleifera leaves on performance, meat characteristics, oxidative stability, and fatty acid profile in growing rabbits. Animals. 2021 Jan 20;11(2): 16 p.

Valacchi G, Belmonte G, Miracco C, Eo H, Lim Y. Effect of combined mulberry leaf and fruit extract on liver and skin cholesterol transporters in high fat diet-induced obese mice. Nutr Res Pract. 2014 Feb 2;8(1):20-6.

Wei M, Mahady GB, Liu D, Zheng ZS, Lu Y. Astragalin, a flavonoid from Morus alba (mulberry) increases endogenous estrogen and progesterone by inhibiting ovarian granulosa cell apoptosis in an aged rat model of menopause. Molecules. 2016 May 21;21(5): 15 p.

Wu P, Li L, Yang H, Zhao W, Chen T, Yan H, Yan Y. Effect of mulberry powder on blood biochemical index and egg quality of laying hens. Feed Ind. 2014;35(7):36-8. Chinese.

Yang JX, Chaudhry MT, Yao JY, Wang SN, Zhou B, Wang M, Han CY, You Y, Li Y. Effects of phyto-oestrogen quercetin on productive performance, hormones, reproductive organs and apoptotic genes in laying hens. J Anim Physiol Anim Nutr. 2018 Apr;102(2):505-13.

Yang SL, Yang RC, Zhou X, Yang SH, Luo LL, Zhu YC, Boonanuntan S. Effects of feeding diets with processed Moringa oleifera stem meal on growth and laying performance, and immunological and antioxidant activities in laying ducks. Poult Sci. 2020 Jul 1;99(7):3445-51.

Zeng B, Luo J, Wang P, Yang L, Chen T, Sun J, Xie M, Li M, Zhang H, He J, Zhang Y, Xi Q. The beneficial effects of Moringa oleifera leaf on reproductive performance in mice. Food Sci Nutr. 2019 Feb;7(2):738-46.

Zhang L, Su S, Zhu Y, Guo J, Guo S, Qian D, Ouyang Z, Duan JA. Mulberry leaf active components alleviate type 2 diabetes and its liver and kidney injury in db/db mice through insulin receptor and TGF-β/Smads signaling pathway. Biomed Pharmacother. 2019a Apr 1;112: 13 p.

Zhang T, Si B, Tu Y, Cui K, Zhou C, Diao Q. Effect of including different levels of moringa (Moringa oleifera) leaf meal in the diet of finishing pigs: Performance, pork quality, fatty acid composition, and amino acid profile. Czech J Anim Sci. 2019b Mar 13;64(3):141-9.

Zhang Y, Yin C, Schroyen M, Everaert N, Ma T, Zhang H. Effects of the inclusion of fermented mulberry leaves and branches in the gestational diet on the performance and gut microbiota of sows and their offspring. Microorganisms. 2021 Mar 15;9(3): 20 p.

Received: October 4, 2021
Accepted: August 2, 2022
Published online: August 22, 2022