Effects of Different Ration of Moringa Oleifera Leaf Powder and Mulberry Leaf Powder on Laying Performance, Egg Quality, Antioxidant Activity, Lipid Metabolism and Organ Index of Laying Hens

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Abstract

Background: The use of moringa oleifera and mulberry as animal feed satisfy not only a nutritional requirement for livestock, but also improve antioxidant status and prevent lipid oxidation. Different feed combinations could produce synergy effect in animals. The combination between moringa oleifera and mulberry may result in additive and/or synergic effects that enhance animal performance. The purpose of this study was to investigate the effect of different ration of moringa oleifera leaf powder (MOLP) and mulberry leaf powder (MLP) on the performance of laying hens.

Methods: A total of 210 37-week-old F1 generation produced from Wenchang Chicken and Rugao yellow chicken were randomly divided into three groups (each group had 5 repetitions; each repetition had 14 chickens). The control group (C0) was fed basal diet, while test I (T1) and test II (T2) were fed basal diet supplemented with 2.5% MOLP + 2.5% MLP and 5% MOLP + 2.5% MLP, respectively. During the experiment, the laying performance, feed conversion, egg quality, internal organ index, antioxidant index and lipid indictors were measured.

Results: The results showed that the supplementation of 5%MOLP and 2.5%MLP significantly decreased laying rate, albumen height, Haugh Unit and eggshell index compared with the Control group *($P<0.05$)*, whereas the supplementation of 2.5%MOLP and 2.5%MLP had no significant effect the above indicators. In the two supplemented groups, yolk colors were significantly increased. A significant decrease of antioxidant activity of serum triglyceride, lipid indictor of liver total cholesterol(T-CHO), and internal organ of abdominal fat index were also observed in 5%MOLP and 2.5%MLP group. RT-qPCR analysis showed that the expression levels of *SOD2* mRNA were significantly increased, while *APOB* mRNA were significantly decreased with the supplementation of MOLP and MLP.

Conclusions: The supplementation of 2.5%MOLP+2.5%MLP in the local chicken diet had no side effect on egg production performance, egg weight and most egg quality, however, it could improve the yolk color and eggshell strength. The combination supplementation of MOLP and MLP may regulate the antioxidant status and lipid metabolism by regulating *SOD2* and *APOB* gene expression. The supplementation of 2.5%MOLP+2.5%MLP was recommended.

Introduction

The concept of “phytogenic feed additives” refers to the natural medicinal product derived from herbs used in livestock nutrition to enhance performance [1]. With the advent of antibiotic use, the phytogenic feed additives have recently received much great attention. Moringa oleifera (MO) is a fast-growing tree originated from Northern India and Africa [2] usually regarded as “miracle tree”. Mulberry has more than 4000 years and made a great contribution to sericulture. As a rich source of protein, moringa oleifera and mulberry can be used as a source of plant protein for livestock and poultry [3, 4]. In addition, moringa oleifera as well as mulberry are both rich in minerals, vitamins, and a variety of biological active ingredients [5], such as quercetin and flavonoids, which are beneficial to human and animal health. A series of studies about the effect of moringa oleifera or mulberry have been carried out in poultry production. Lu et al. [6] proposed that supplementation with 5% moringa oleifera leaf (MOL) could improve Hy-Line Grey hens laying performance and egg quality. A study by Cui et al. [7] stated that supplementation with 1.56% MOL could improve Arbor Acres broilers muscle quality including PUFA contents, oxidative stability and color of breast muscle. Ashour et al. [8] findings indicated that the inclusion of moringa oleifera seeds in Japanese quail diet could significantly increase egg production and egg quality and lower some blood biochemical components. Several studies have also presented the beneficial effects of mulberry leaves on livestock and poultry. Studies in pigs showed that the supplementation level of mulberry leaf powder less than 12% would improve meat quality [9]. The flavonoid and resveratrol in mulberry leaf could enhance digestibility of nutrients, the utilization of nutrients and energy in sheep [10]. The application of mulberry has also improved performance, quality of products and oxidant activity in poultry. Olteanu et al. [11] found that mulberry leaf supplementation in dietary could improve breast meat quality in broiler and Chen et al. [12] suggested that mulberry leaf extract-based dietary supplementation could modulate antioxidative of laying hens. Taken the above literatures together, as a feed or feed additive, moringa oleifera or mulberry has been widely used in the poultry and has been reported to significantly improve performance and product quality.

Different feed combinations supplementation in base diet could produce synergy effect in animals. In an in vitro study, Rofiq et al. [13] demonstrated that the combination of clove and orange peel in dairy total mixed ration play a role in antagonistic effect in decreasing digestion. Martono et al. [14] reported that the combination of different feed supplements could increase feed efficiency for dairy cattle. Liu et al. [15] reported that supplementation of 75% whole silage corn and 25% peanut vine in Small Tail Han Sheep had the optical combination effect on the feed to meat ration. However, no relevant literature has been reported about the combination effect of moringa oleifera and mulberry.

As the economy grows and living standards improves rapidly, people seek more diverse diets. China local chicken is becoming more widespread in the past 20 years, holding a 42% share of the market and China consumers display a strong preference for local breeds of chickens [16]. There is a need to investigate the nutrient requirements of China local chicken. The purpose of this study was to seek the appropriate ration of moringa oleifera and mulberry in China local chicken diet and provide reference for utilization plants in poultry.

Materials And Methods

Animals, Experimental Design and Treatments

Moringa oleifera leaf powder (MOLP) were obtained from Yunnan Dayaoshan Trading Co.,LTD.(Yunnan, China), and mulberry leaf powder (MLP) were obtained from Danyang Tianyuan Shengshu Ecological Park Co.,LTD. (Zhenjiang, China). Moringa oleifera leaf and mulberry leaf powder were picked, dried, crushed, sifted and stored. The contents of crude protein, crude fat and crude fiber in MOLP were 26.95%, 5.76% and 19.26%, respectively. The contents of crude protein, crude fat and crude fiber in MLP were

13.79%, 1.98% and 24.89%, respectively. The basic diet is a corn-soybean meal diet provided by China Oil and Foodstuffs Corporation. Our experimental animals were provided by Jiangsu Institute of Poultry Science. A total of 210 birds of F1 generation produced from Wenchang Chicken and Rugao Yellow Chicken were randomly assigned to three groups each group consisted of 5 replicates (14 birds each replicate). These groups are: C0. basal corn-soybean meal, T1. feed supplemented with 2.5% MOLP +2.5% MLP, T2. 5% MOLP +2.5% MLP. The experiments lasted 7 weeks including 1week adaptation. The ingredients and chemical composition of the feed are shown in Table 1.

Management of Experimental Birds

All animal care and experimental procedures were approved by the Institutional Animal Care and Use of Committee of Jiangsu University of Science and Technology. Our experiment was carried out in Poultry Institute, Chinese Academy of Agricultural Sciences. All hens were raised in single cage and ad libitum, with the photoperiod regime was 16L:8D throughout the study.

Sample Collection and Analytical Determination

Laying Performance

Daily egg production was monitored during the trail, average egg weight and feed intake were recorded weekly. Laying rate is expressed as average hen-day production, calculated from the total number of eggs divided by the total number of days. Feed intake was recorded weekly and their conversion was determined.

Egg Quality

Freshly laid eggs were collected at the end of 6^{th} week. The internal and external egg quality of 6 randomly selected eggs per group (6 eggs/ replicate) were measured. The eggs were stored at room temperature before measurement. The length and width of the eggs were measured using the electronic digital caliper and the egg shape index (ESI) were calculated (length /width ×100). Eggshell thickness (EST) was measured using the eggshell thickness tester ESTG-1(ORKA Co. Ltd.,) at the blunt, equatorial, and sharp regions to obtain an average value. Eggshell color (ESC) was measured using the spectrophotometer CM-2300D (MINOLTA Co. Ltd.,) and three traits were recorded: lightness of eggshell L*, redness of eggshell a* and yellowness of eggshell b*. Eggshell Strength (ESS) was evaluated using the EggShell Force Gauge EFR-01(ORKA Co. Ltd.,). Egg weight (EW), albumen height (AH), Haugh unit (HU), and yolk color (YC) were measured using the Egg Multi Tester EA-01(ORKA Co. Ltd.,). Then, the yolk weight (YW) and yolk rate (YR) was calculated. Eggshell weight (ESW) is weighed after natural drying.

Sample collection

Thirty hens (2 hens/per replicate, 10 hens per group) were selected after 12 hours fasting at the end of 6^{th} week. Blood were collected from wing vein. Serum were obtained by centrifugation at 4000r/min for 10min of the blood and stored at -20°C. Then the hens were humanely killed by carbon dioxide overdose, and their internal organ including heart, liver, spleen, lung, kidney, abdominal fat were removed and measured, their index was calculated by the following formula: internal organ index% = (internal organ weight/body weight) × 100. Specially, liver tissues were collected and stored at -80°C until assayed for antioxidant or lipid indicators and their related genes expression analysis.

Antioxidant and lipid indicators

Livers samples were homogenized with saline to make a 10% homogenate with 0.9% sodium chloride buffer with tube embed in ice and centrifuged at 4000 rpm at 4°C for 10 min. The serum and liver supernatant were used to measure MDA, SOD, T-AOC, GSH, TG, T-CHO, HDLC, LDLC by ELISA method, using commercial kits bought from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction

Total RNA of liver was extracted using Trizol Reagent (TaKaRa Biotechnology, Dalian, Liaoning, China). Quality and integrity of RNA was assessed by Nanodrop ND- 2000c spectrophotometer (Thermo Scientific, Camden, NJ). The reverse transcription was carried out according to Takara reverse transcription kit protocol (Perfect Real Time, PrimeScriP™ TaKaRa, China). The reaction conditions of reverse transcription were as follows: reaction at 37 °C for 15 min, deformation at 85 °C for 15 s, and finally cooling to 4 °C. The real-time quantitative polymerase chain reaction was carried out using the SYBR Premix Ex Taq II kit (TaKaRa,Dalian, China) in an ABI 7300 fluorescence quanti-tative polymerase chain reaction instrument (Applied Biosystems, Foster City, CA). The 20μL reaction system included 10 μL of SYBR Premix Ex Taq buffer, 0.4μL each of forward and reverse primers and ROX,1μL of cDNA template, and 7.8 μL of distilled water. The real-time polymerase chain reaction cycling conditions were as follows: 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 31 s. The relative mRNA expression was determined using *β-actin* as an internal reference gene. The significance and correlation of quantitative results were analyzed using $2^{-\Delta\Delta ct}$ [17]. Primer sequences are shown in Table 2.

Results

Production performance

No sick or dead chickens were found in each group during the experiment. As can be seen from Table 3, compared with the control group(C0) and test I (T1), the laying rate of test II (T2) decreased significantly ($P < 0.05$). There was no significant difference in average egg weight among the groups ($P > 0.05$). The feed conversion was 2.51, 2.42 and 2.47 in C0, T1 and T2 groups, respectively. There was significant difference in feed conversion among the groups ($P < 0.01$). The average daily feed intake was 91.22, 84.63 and 82.07 g per hen in C0, T1 and T2 groups, respectively.

Egg Quality

It can be seen in Table 4 that T1 and T2 showed significantly higher eggshell strength compared to C0 group ($P<0.05$). But the albumen height (AH) and the haugh unit (HU) decreased with the supplementation of moringa oleifera leaf powder (MOLP) and mulberry leaf powder (MLP), and there is a significant decrease of AH in T2 group ($P<0.05$). The yolk color value significantly increased as the increase of MOLP and MLP supplementation levels in the diets ($P<0.05$). The rest of egg quality index was no significant difference among groups.

Serum antioxidant activity and lipid indicators

As shown in Table 5, no significant differences were observed among groups in terms of serum antioxidant index such as superoxide dismutase (*SOD*), malondialdehyde (MDA) and total antioxidant capacity (T-AOC)（$P>0.05$）.Supplementation MOLP and MLP could reduce the concentrations of total cholesterol (T-CHO), high-density lipoprotein (HDLC) and low-density lipoprotein (LDLC) in serum, but there is no significant difference among groups（$P>0.05$）. Triglycerides (TG) decreased significantly when hens fed 5% MOLP and 2.5% MLP compared to C0 and T1 groups（$P<0.05$）.

Liver antioxidant activity and lipid indicators

As presented in Table 6, no significant effects on antioxidant activity were detected among all treatments（$P>0.05$）. There was no significant difference of the lipid indicators of TG, HDLC and LDLC among groups. Compared with C0 group, CHO activity in the T2 group significantly decreased（$P<0.05$）, and a linear decrease in CHO value was detected as supplementation level of MOLP increased.

Expression level of antioxidant and lipid related gene

mRNA expression levels of *CAT, Nrf2, SOD1, SOD2, ACC* and *APOB* in liver were shown in Figure 1**.** *CAT, Nrf2, SOD1* and *ACC* mRNA showed no different significant among groups, but the *ACC* mRNA level decreased in the treatment groups. Treatment with MOLP and MLP significantly reduced the expression level of *APOB* mRNA （$P<0.05$）, and significantly increased the expression level of *SOD2* mRNA.

Internal organ index

As shown in Table 7, dietary supplementation of MOLP and MLP had no significant effects on liver, lung, kidney, muscular stomach, glandular, duodenum index ($P>0.05$）. Heart index significantly decreased in T2 group compared with C0 and T1 group. Both MOLP and MLP could significantly reduce the abdominal fat index （$P<0.05$）.

Discussion

The aim of our study was to seek the proper supplementation ration of MOLP and MLP in China local chickens. Previous studies from our team showed that the optimal supplementation level of MLP should less than 4% in the basal diet in Yangzhou goose [18]and Blue eggshell chickens [19]. Considering the chicken used in current experiment was the China local chicken, we designed the supplement ration of

MLP as 2.5%. Previous studies showed that the high supplementation of MOLP had side effects on the laying performance of chickens [6, 20],therefore, the final highest total supplementation level of MOLP and MLP was 7.5% in basal diet. Our study showed that the different ration of MOLP and MLP affected the performance in China local chickens. The supplementation of 5%MOLP and 2.5%MLP had side effects on laying performance, which is consistent with previous studies [6] which showed that the higher supplementation level of MOLP in hens, the higher adverse effect had. A study by Cui et al. [7], showed that the recommended supplementation of MOLP to broiler feed dietary was 1.56%. The fiber content in MOLP and MLP was 19.26% and 24.89%, respectively. Generally, the dietary fiber was considered a diluent of poultry diet and the optimum supplementation should not exceed 3% in broiler feed [21]. However, dietary fiber was also proven to improve gizzard weight, amylase activity and bile acid, which are beneficial to intestinal health [22]. A healthy intestinal indirectly affect the laying performance through the energy saving for the host that translates to egg production [23]. In current study, T1 group with supplementation of 2.5%MOLP and 2.5%MLP increased feed conversation but had no adverse effect on laying performance. Therefore, supplementing feed with an appropriate ration of MOLP and MLP could be useful in supporting bird health and productivity.

T1 and T2 groups both significantly improved yolk color, but T2 group had adverse effect on albumen quality. This observation is consistent with previous works showing that supplementation of MOLP in Sasso broiler breeder hens and Rhode Island Red hen improved yolk color [24, 25]. Yolk color is mainly composed ofα-carotene,β-carotene, lutein and carotenoids [26], and was influenced by many factors such as breed, age, management and feed, in all the same above situations with feed being the most important. Both moringa oleifera and mulberry are rich inβ-carotene, the content in leaves was 13.48–18.50 mg/100 g [27, 28] and 7.44 mg/100 g [29], respectively. The darker yolk color eggs are highly popular in China consumers. This indicated that supplementation MOLP and MLP can have beneficial effects on yolk color and egg market.

Though supplementation of 5%MOLP and 2.5%MLP could significantly decrease serum TG level and liver T-CHO level*($P < 0.05$)*, from all the lipid metabolism trends, supplementation of MOLP and MLP could be able to decrease the lipid indicators. Furthermore, treatment with MOLP and MLP significantly decrease *APOB* mRNA expression level. Report by Alnidawi et al. [30] showed that supplementation of moringa oleifera decreased chicken serum T-CHO, TG, HDL and LDL. A study by Krauss RM et al. [31] showed that some antioxidant substances could reduce the breakdown of APOB. In chicken, APOB plays a role in transporting lipid to the ovary for yolk deposition [32]. It was evident from the findings of lipid indicators in serum and liver at current study that treatment with MOLP and MLP decreased these indicators but failed to achieve statistical significance. Longer experiment observation times may be needed in future. It has been verified that plant-derived feed could activate the SOD2 signal pathway to eliminate reactive oxygen species (ROS) level. Our study showed that supplementation different ration of MOLP and MLP had no significant effect on the antioxidant enzyme activities in serum and liver. But treatment with MOLP and MLP could significantly increase *SOD2* mRNA expression level in liver and only slight increase *SOD2* activity in liver. *SOD2* has been shown to play a role in eliminating reactive oxygen species (ROS) under oxidant stress and are involved in many downstream signaling pathways, such as *FOXO3*, *SIRT3*, and *STAT* signaling pathway [33, 34]. The reasons about the difference between oxidant activity and mRNA expression may be a short supplementation time does not produce effects in the synthesis of antioxidant enzymes. Thus, further treatment studies with a long-term supplementation of MOLP and MLP are recommended.

Study by Wen et al. [35] showed that flavonoid-rich mulberry leaf could prevent ROS production and up-regulate the expression of antioxidant related genes including *SOD2* and *Nrf2*. Report about moringa oleifera leaf have shown that phenolic content rich in the leaf have potential antioxidant activity and afford protection against oxidative damage [36] and a series of nutrition data revealed that moringa oleifera is widely involved in the antioxidant status in different species [5, 37, 38]. In addition, a number of previous studies have analyzed the combination of mulberry and other phytogenic additive and found the combination effect between them. A study in tilapia juvenile showed that combination 30% of mulberry leaf meal and 0.4% bamboo charcoal additive improved blood lipid metabolism and antioxidant activity [39]. The effective ration of mulberry leaf extract and mulberry fruit was 2:1 in the obese mice meal ameliorated obesity and obesity-related metabolic stressors through decreasing oxidant stress [40, 41]. From our findings and the previous studies, we can draw some implications that combination of MOLP and MLP increased antioxidant status and had no adverse effect in later peak laying hens.

Conclusion

To our knowledge, this is the first report to study the combination effect of MOLP and MLP on chicken. Results in current study demonstrated that supplementation of MOLP and MLP could significantly decrease feed conversion and abdominal adipose percentage, while it could improve yolk color and eggshell strength. The decrease in serum TG level and liver T-CHO level were significant in 5%MOLP + 2.5%MLP group but did not significance in 2.5%MOLP + 2.5%MLP group. Changes of antioxidant activity and lipid metabolism could be modulated by *SOD2* and *APOB* gene expression. However, the 5%MOLP + 2.5%MLP supplementation level had a side effect on laying performance. Taken together with the production performance and physiological indexes, the suitable addition and combination level was 2.5%MOLP + 2.5%MLP in China local chicken.

Statistical Analysis

All data were analyzed using SPSS 20 statistical software. One-way analysis of variance (ANOVA) followed by Duncan`s multiple comparison test was used to evaluate different means among treatments. Data were assumed to be statistically significant at $P < 0.05$.

Abbreviations

MOLP: Moringa oleifera leaf powder

MLP: mulberry leaf powder

CHP: Calcium hydrogen phosphate

CAT: catalase

Nrf2: nuclear factor erythroid 2-related factor 2

APOB: Apolipoprotein B

VTG Ⅱ: Vitellogenin II

SOD2: superoxide dismutase 2

ACC: acetyl coenzyme A carboxylase

FAS: fatty acid synthase

PPARα: peroxisome proliferator activated receptor alpha

EW: egg weight

ESC: eggshell color

ESW: Eggshell weight

EST: eggshell thickness

YW: yolk weight

ESS: eggshell strength

AH: albumen height

HU: Haugh unit

YC: yolk color

ESI: eggshell index

YR: yolk rate

MDA: Malondialdehyde

SOD: Superoxide dismutase

T-AOC: Total Antioxidant Capacity

GSH: glutathione

TG: triacylglycerol

T-CHO: total cholesterol

HDLC: high-density lipoprotein

LDLC: low-density lipoprotein

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current research are included with the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TL, MMS, PW, and WGZ were responsible for the study design. TL and MMS performed data analysis. TL, MMS, HLH, SLZ, PG and QRH collected the data. TL and MMS wrote the manuscript. TL, MMS, and PW participated in the interpretation of the results and review of the paper. All authors read and approved the final manuscript.

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Tables

Table 1.

Proportion (%) of ingredients used for formulating experimental diets

Basal Diets	**Compositions**		
Ingredient (%)	C0	T1	T2
Corn grain	64.420	63.092	62.605
Soybean	23.520	21.440	19.975
Shell powder	6.700	6.700	6.700
Salt	0.300	0.300	0.300
CHP	0.878	0.810	0.780
Stone powder	2.107	1.75	1.635
Zeolite powder	1.632	0.459	0.053
Choline chloride	0.170	0.170	0.170
Methionine	0.116	0.122	0.125
Lysine	0.100	0.100	0.100
Vitamin premix	0.050	0.050	0.050
Phytase	0.007	0.007	0.007
Total %	100	100	100
Nutrient levels[1]	C0	T1	T2
ME(MJ/kg)	11.0874	11.0870	11.0872
Crude protein (%)	16.000	16.000	16.000
Crude fibre (%)	2.418	3.075	3.158
Lys (%)	0.785	0.785	0.793
Met (%)	0.370	0.370	0.370
Calcium （% DM）	3.35	3.35	3.35
Phosphorus（% DM）	0.32	0.32	0.32

MOLP: Moringa oleifera leaf powder; MLP: mulberry leaf powder; CHP: Calcium hydrogen phosphate C0: basal diet; T_1 and T_2 basal diet supplemented with 2.5% MOLP +2.5%MLP and 5%MOLP+2.5%MLP, recpectively.

[1]Calculated value.

Table 2.

Primer sequences used in current study

Gene	Primer sequence（5’-3’）	Product size（bp）	Tm（°C）
β-Actin	F: CAGCCATCTTTCTTGGGTAT	167	59.1
	R: CTGTGATCTCCTTCTGCATCC		59.1
CAT	F：TGCAAGGCGAAAGTGTTTGA	158	58.9
	R: CCCACAAGATCCCAGTTACCT		59.1
NRF2	F: AACGCACCAAAGAAAGACCC	147	58.9
	R: ACTGAACTGCTCCTTCGACA		58.9
SOD1	F: TACCGGCTTGTCTGATGGAG	172	59.1
	R: TCCTCCCTTTGCAGTCACAT		58.9
SOD2	F: AGAGGAGAAATACAAAGAGGCG	245	57.8
	R: AGCCTGATCCTTGAACACCA		58.9
ACC	F: AGACGAGCTCCTTGGTGAAA	217	58.9
	R: GAAGCCACAGTGAAATCCCG		59.2
APOB	F: ACGGGAACAGCAGATTCTCA	225	59
	R: TGTTCCATCCTGAGTGCTGA		58.6

CAT: catalase; Nrf2: nuclear factor erythroid 2-related factor 2; *SOD*: superoxide dismutase; *ACC*: acetyl coenzyme A carboxylase; *FAS*: fatty acid synthase; *PPARα*: peroxisome proliferator activated receptor alpha; *APOB:* apolipoprotein B; *VTGⅡ: Vitellogenin II;*

Table 3.

Effect of Moringa oleifera leaf powder and mulberry leaf powder on performance of laying hens.

Item	Supplement		
	C0	T1	T2
Laying rate (%)	78.91%±0.014^{a}	76.94%±0.012^{a}	73.64%±0.017^{b}
Average egg weight(g)	46.06±0.74	45.61±1.07	44.93±0.98
Average daily feed intake (g/bird /d)	91.22	84.62	82.07
Feed conversion (g of feed/g of egg)	2.51^{a}	2.42^{b}	2.47^{c}

^{a,b,c}Values with different characters in superscripts were different*(P<0.05)* in the same row

Table 4.

Effect of Moringa oleifera leaf powder and mulberry leaf powder supplementation on Egg quality

		C0	T1	T2	S.E.M	MO	Linear	Quadratic
EW(g)		45.68	46.25	45.58	0.35	0.695	0.407	0.407
ESC	L	74.09	73.63	74.64	0.36	0.519	0.820	0.263
	a	9.09	9.23	8.79	0.24	0.744	0.785	0.473
	b	21.11	20.94	20.13	0.34	0.460	0.352	0.407
ESW(g)		4.30	4.52	4.35	0.06	0.256	0.361	0.169
EST(mm)		0.36	0.37	0.37	0.003	0.605	0.337	0.779
YW(g)		14.71	14.85	14.73	0.13	0.890	0.820	0.672
ESS(kgf)		3.54^{a}	3.77^{b}	3.72^{b}	0.07	0.419	0.214	0.664
AH(mm)		4.23^{a}	4.24^{a}	3.74^{b}	0.08	0.011	0.070	0.015
HU		69.17^{a}	67.74^{a}	62.74^{b}	0.77	0.001	0.004	0.014
YC		7.27^{a}	9.92^{b}	10.70^{c}	0.20	<0.001	<0.001	0.043
ESI		1.31^{ab}	1.3^{a}	1.29^{b}	0.005	0.098	0.461	0.043
YR		0.32	0.32	0.32	0.002	0.943	0.945	0.738

EW: egg weight; ESC: eggshell color; ESW: Eggshell weight; EST: eggshell thickness ;YW: yolk weight; ESS: eggshell strength ; AH: albumen height ; HU: Haugh unit; YC: yolk color ; ESI: eggshell index; YR: yolk rate ;

^{a,b,c}Values with different characters in superscripts were different*($P<0.05$)* in the same row

Table 5.

Effect of dietary on Serum antioxidant and lipid indicators of hens

Item	Supplement			P-value			
	C0	T1	T2	S.E.M	MO	Linear	Quadratic
MDA（nmol/ml）	12.39	12.31	12.05	0.242	0.227	0.231	0.210
SOD（U/ml）	16.04	15.79	16.12	0.36	0.948	1.000	0.746
T-AOC（U/ml)	0.54	0.54	0.57	0.03	0.913	0.772	0.759
GSH（U/ml)	15.11	11.19	14.10	1.60	0.602	0.561	0.416
TG（nmol/gprot）	14.14^{a}	13.60^{a}	10.42^{b}	0.701	0.06	0.08	0.097
T-CHO（nmol/gprot）	5.28	4.72	4.45	0.26	0.44	0.21	0.830
HDLC（nmol/gprot）	2.47	2.08	1.73	0.18	0.266	0.123	0.595
LDLC（nmol/gprot）	0.86	0.77	0.61	0.046	0.077	0.049	0.249
GLUCOSE（nmol/gprot）	18.01	16.69	17.92	0.701	0.715	0.742	0.457

MDA: Malondialdehyde; SOD: Superoxide dismutase; T-AOC: Total Antioxidant Capacity; GSH: glutathione; TG: triacylglycerol; T-CHO: total cholesterol; HDLC: high-density lipoprotein; LDLC: low-density lipoprotein;

^{a,b}Values with different characters in superscripts were different*($P<0.05$)* in the same row

Table 6.

Effect of dietary on antioxidant capacity evaluation and lipid indicators of livers of hens

Item	Supplement			P-value			
	C0	T1	T2	S.E.M	MO	Linear	Quadratic
MDA（nmol/ml）	4.72	4.38	4.97	0.34	0.801	0.999	0.509
SOD（U/ml）	9.37	10.56	9.45	0.73	0.766	0.788	0.501
T-AOC（U/ml)	3.22	2.96	2.95	0.11	0.515	0.257	0.878
GSH（U/ml)	123.45	124.70	140.41	6.45	0.519	0.446	0.395
TG（nmol/gprot）	4.70	4.74	3.42	0.30	0.123	0.217	0.097
T-CHO（nmol/gprot）	0.76^{a}	0.65^{a}	0.59^{b}	0.032	0.078	0.029	0.615
HDLC（nmol/gprot）	0.08	0.06	0.06	0.006	0.288	0.129	0.698
LDLC（nmol/gprot）	0.41	0.38	0.38	0.012	0.641	0.350	0.999

MDA: Malondialdehyde; SOD: Superoxide dismutase; T-AOC: Total Antioxidant Capacity; GSH: glutathione; TG: triacylglycerol; T-CHO: total cholesterol; HDLC: high-density lipoprotein; LDLC: low-density lipoprotein;

[a,b]Values with different characters in superscripts were different*(P<0.05)* in the same row

Table 7.

Effect of dietary on Organ development of hens

Item	Supplement			P-value			
	C0	T1	T2	S.E.M	MO	Linear	Quadratic
Heart index (%)	0.530^{a}	0.446^{b}	0.518^{ab}	0.016	0.053	0.027	0.033
Liver (%)	1.939	1.873	1.880	0.041	0.785	0.504	0.864
Spleen (%)	0.110^{a}	0.087^{b}	0.102^{ab}	0.004	0.065	0.108	0.081
Lung (%)	0.400	0.394	0.441	0.013	0.306	0.409	0.194
Kidney (%)	0.480	0.465	0.464	0.019	0.936	0.722	0.963
Abdominal adipose (%)	5.50^{a}	3.73^{a}	2.95^{b}	0.412	<0.001	0.010	0.673
Muscular stomach (%)	0.364	0.361	0.363	0.007	0.984	0.950	0.867
Glandular stomach (%)	0.200	0.211	0.206	0.008	0.931	0.745	0.745

[a,b,c]Values with different characters in superscripts were different*(P<0.05)* in the same row

Figures

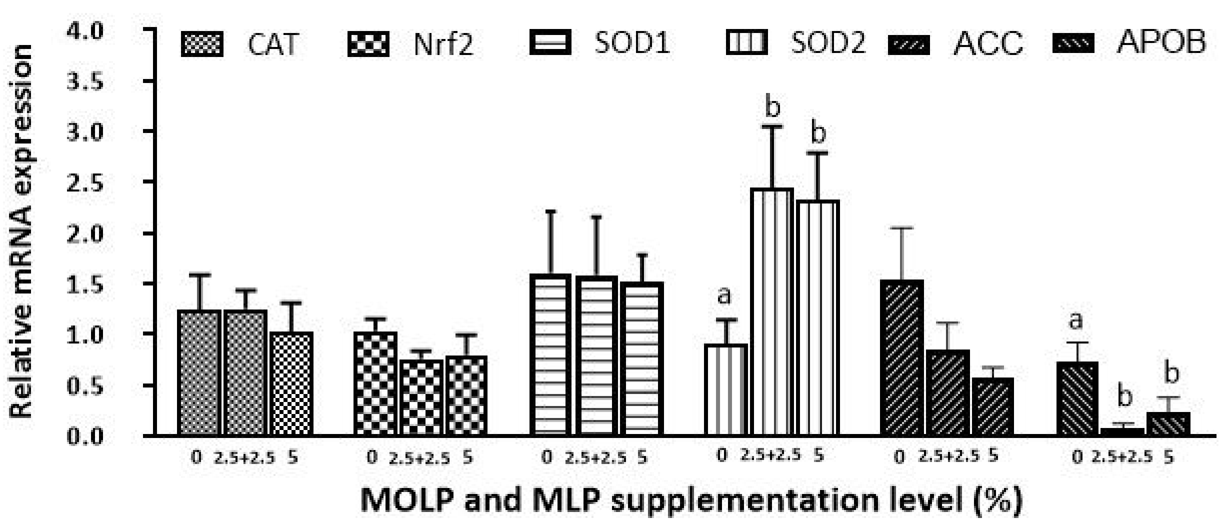

Figure 1

Expression level of antioxidant and lipid related gene