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Effects of Chinese Herbal Medicine Additives on Laying Performance, Egg Quality and Yolk Nutrition of Laying Hens During Late Laying Period

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HW presented the concept and planned methodology. KD and HW supervised and administered the project. KD, HW and JL performed formal analysis. HW, BM, FZ and JL curated data. BM, FZ and JL did investigation. HW, BM and JL wrote the original draft. HW and FZ arrnaged resources. JL validated the study. HW and KD reviewed and edited the manuscript. KD and HG procure funds. HG modifies language.

Key words
Chinese herbal medicine, Late laying period, Laying performance, Egg quality, Amino acid

ABSTRACT

We investigated the effects of different proportions of Chinese herbal medicine (CHM) additives on laying performance, egg quality and yolk nutrition of aged laying hens. We utilized a mixture of 8 components that included the Fabaceae family members *Radix astragali* and *Sophora flavescens* as well as dandelion, pine needle powder, marigold, rosemary, Shenqu (a leavened mixture) and *Gardenia jasminoides*. Our study groups included 2000 healthy 350 days old laying hens that were randomly divided into 4 groups and standard feed was supplemented with 0 (control), 0.5, 1 and 1.5 % additive. The average laying rates for the test groups increased by 0.65, 1.68 and 0.54 %, respectively ($P > 0.05$) while egg breakage decreased by 14.19, 20.34 and 18.64% ($P < 0.05$) while feed: Egg ratios decreased by 3.46, 5.63 and 0.87, respectively. Yolks were a deeper yellow in the presence of the additives and yolk viscosity increased significantly ($P < 0.05$) by 40, 77 and 74%, respectively. Compared with the control group, crude fat, methionine, isoleucine, valine, aspartic acid and proline, Crude fat increased by 6.17, 7.18 and 7.09 respectively ($P < 0.05$). This study provides evidence that the overall laying performance of aged hens was improved with CHM additions and the effects were dosage-dependent with an optimal dosage of 1 % w/w.

INTRODUCTION

The physiological, metabolic and reproductive performance of laying hens gradually declines with age resulting in decreased production, egg quality and increased mortality (Hao *et al*., 2021). Therefore, the late laying stages are crucial for continued production and can be theoretically extended by prolonging the laying cycle and optimizing nutritional requirements (Zhang *et al*., 2021). A cost-effective approach is the use of green feed additives that primarily include organic acids, oligosaccharides, Chinese herbal medicine (CHM), plant extracts, microecological agents, lysozyme and other novel feed additives. CHM are a traditional Chinese and essential source of green feed additives. These additives have been administered to aging laying hens and were successful in improving laying rate, egg quality and nutritional value and health of the animals (Wen *et al*., 2019). For example, inclusion of 1-2% of the CHM additives *Lycium barbarum* (gogi berry), *Cuscuta* spp. (dodders) and *Rehmannia glutinosa* (Chinese foxglove) improved egg yield and quality and alleviated decline in laying hen ovarian functions (Zhang *et al*., 2022). The addition of a 0.25% *Ganoderma lucidum* (a Basidiomycete fungus) powder to the feed of 72 weeks old laying hens reduced mortality, egg-breakage, serum and egg low-density lipoprotein cholesterol and triglycerides and increased high-density lipoprotein cholesterol (Li *et al*., 2017). Egg thickness and strength in these older animals was also increased using the simple feed supplements mint (Lokaewmanee *et al*., 2014), rosemary (Bolukbasi *et al*., 2018), garlic (Abdelqader and Al-Fataftah, 2013) and inulin (Ufadar *et*

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al., 2018). CHMs are also rich in minerals, plant pigments and trace elements. Plant pigments can be absorbed by the hen and transferred to the egg ovaries to improve egg yolk color (Guo *et al*., 2022).

In the current study, we examined the effects of traditional Chinese medicines to improve hen laying performance. We utilized a mixture of 8 components that included the Fabaceae family members *Radix astragali* and *Sophora flavescens* as well as dandelion, pine needle powder, marigold, rosemary, Shenqu (a leavened mixture) and *Gardenia jasminoides*. This mixture was added to feed to explore dosage effects on laying performance, egg quality and nutritional composition using caged laying hens. These data will provide reference materials for the application of TCM in aging laying hens.

MATERIALS AND METHODS

Materials

Radix astragali, Sophora flavescens, dandelion, pine needle powder, marigold, rosemary, Shenqu*, Gardenia jasminoides* were provided by Zhang Zhongjing National Medical Center. After drying and crushing, the components were mixed in the following proportions; 2 : 5 : 1 : 3 : 1 : 1 : 0.5 and this mixture was used as the feed additive for the hens.

Instruments and equipment

Electronic balance (FA1004, Shanghai Liangping Instruments), Soxhlet extractor (Zhengzhou Xinyuan Glass Instruments), dissolver (SPH120, Jinan Alva Instruments), spectrophotometer (TB-1810U, Beijing Purkinje General Instrument), Digital rotary viscometer (NDJ-79B, Shanghai Changji Geological Instrument), Liquid chromatograph-mass spectrometer (Agilent 1260, Agilent Technologies, San Diego, CA, USA).

Animals

The laying hens used in the experiment were provided by Shangshui County for specialized farming cooperatives. 2000 healthy Roman laying hens with similar weights in the same henhouse were randomly divided into 4 groups with 5 replicates in each group. The control group was fed with a basic diet (Table I) and groups I, II and III were fed the basic diet supplemented with 0.5, 1 and 1.5% of the CHM for 10 days and then fed with elemental diets for 7 days and again with CHM for 27 days. The animals were fed once each in the morning and evening and water was available *ad libitum*. The animals were exposed to light for 16.5 h per day and feces were cleared every 2 days. Routine immunizations, regular ventilation and disinfection were carried out to ensure environmental hygiene in the henhouse. All the experimental procedures involved in this study were approved by the Animal Care and Ethics Committee of Xinyang Agricultural and Forestry University (Approval No: 2022-024, Xinyang, China).

Table I. Composition and nutrient levels of the basal diet.

Ingredients	Content %	Nutrient components	Content %
Corn	62	ME (MJ/Kg)	12.82
Wheat bran	3.78	CP	17.20
Soybean meal	15	P	0.42
Cottonseed meal	6	Ca	3.08
Rapeseed meal	4	Lys	0.71
Lys	0.02	Met	0.46
Met	0.2		
Limestone	8		
Premix	1		

Per kilogram premix contained the following: VA 7715IU; VD3: 2755IU; VE 8.8IU; VK32.2mg; VB120.01mg; Riboflavin: 4.41mg; Pantothenic acid (d-Calcium pantothenate): 5.51mg; Nicotinic acid: 19.8mg; Folic acid 0.28mg; Biotin 0.1mg; Mn: 50mg; FeSO4: 25mg; CuSO4 2.5mg; Zn80mg; I: 1.0mg; Se0.15mg; Choline 500mg. Metabolic energy were calculated values.

Sample collection

At the end of the feeding trial, 10 eggs were randomly selected from each replicate and part were stored at 4°C for egg quality determination. The other part was separated from the egg whites and yolks and used to analyze the yolk conventional nutrient and amino acid content.

Determination of laying performance

The mental states, feed intake and feces weights of the laying hens were observed every day and used to calculate the average laying rate and the feed: egg ratios as well as the numbers of broken eggs in each group.

Laying rate = total number of eggs per day/ total number of layers in the experimental group × 100

Feed/egg ratio = feed intake/egg weight × laying rate

Breaking rate = number of broken eggs/ total number of eggs

Determination of egg quality

(1) Egg weight: 25 unbroken eggs of the same size were selected from each group and weighed to 0.01 g.

(2) Egg shell weight: the egg was broken on a plate and the egg shell was removed and weighed to 0.01 g.

(3) Egg shape index: the longitudinal (vertical

diameter) and transverse diameter (transverse diameter) of each egg were measured with a Vernier caliper to 0.02 mm. The egg vertical diameter/egg transverse diameter was the egg shape index.

(4) Yolk index: the broken eggs (from 3, above) were used and yolk diameters were measured with a vernier caliper; the needle was inserted from the top of the yolk center and the height of the needle insertion was taken as the yolk height to 0.02 mm. Yolk height/yolk diameter was the yolk index.

(5) Yolk color: the chromaticity of yolks were compared with reference to the Roche colorimetric fan and color grades were averaged for each group.

(6) Yolk viscosity: yolks were separated and a digital viscometer was used to measure viscosity.

Analysis of routine nutritional components of egg yolk

Moisture content was determined by the direct drying method according to GB 5009.3-2016. Ash content was determined by the high-temperature cautery method according to GB 5009.4-2016. Crude protein was determined by the semi-micro Kjeldahl method according to GB-5009.5-2016. Crude fat content was determined by the Soxhlet extraction method according to GB 5009.6-2016. Ca and P levels were determined according to the requirements in GB 5009.92-2016 and GB 5009.87-2016, respectively.

Analysis of amino acids in egg yolk

Acidolysis of egg yolks was carried out in bottles containing 100 mg sample and 10 mL 6 M HCl/ 1% phenol that were evacuated with N_2 gas and sealed. The bottles were then maintained at 110°C for 22 h. Water was then added to 50 mL and the liquid was evaporated under an N_2 stream at 95 ℃ to a volume of 1 mL and 1 mL 0.01 M HCl was then added and the solutions were membrane-filtered.

Samples were derivatized online using the Agilent 1260 instrument using the following reagents: Primary amino acids, o-phthalaldehyde; secondary amino acids, fluorene methoxycarbonyl chloride. Separations were conducted using a Zorbax Eclipse AAA column (4.6 × 50 mm, 3.5 µm) and eluates were detected by fluorescence using excitation = 266 nm and emission = 305 nm) except for proline. Other amino acids were detected at 338 nm. The mobile phase consisted of A: 40 mM NaH_2PO_4 pH 7.8 and B: acetonitrile/methanol/water = 45 : 45 : 10. Components were eluted with a gradient (Table II).

Data processing and statistical analysis

Preliminary data were sorted using Excel 2010 and statistically analyzed using SPSS 21.0 software (IBM, Chicago, IL, USA). All data in this study were expressed as mean ± standard deviation and the differences between groups were tested using one-way ANOVA.

Table II. Gradient elution process.

Time	A/% (40 mM phosphoric acid buffer)	B/% Methanol: Acetonitrile: Water (45:45:10)
0	100	0
1	100	0
23	46	57
27	0	100
34	0	100
40	100	0
41	100	0

RESULTS

Effects of CHM additives on egg-laying performance

The addition of our CHM additives to feed improved the overall laying performance of the caged hens. The average laying rates for test groups I, II and III were 0.65, 1.68 and 0.54 % higher than controls, respectively but not significantly ($P > 0.05$) different. The numbers of broken eggs for these test groups significantly ($P < 0.05$) decreased by 14.19, 20.34 and 18.64 %. The feed: egg ratios decreased by 3.46, 5.63 and 0.87 %, respectively but the values were significant ($P < 0.05$) only between groups I, II and controls ($P > 0.05$) (Table III).

Table III. Effect of Chinese herbal medicine additives on production performance of layers.

Items	Control group	Group I	Group II	Group III
Egg production rate (%)	87.43±0.42^a	88.00±0.51^a	88.90±0.54^a	87.90±0.60^a
Broken egg rate (%)	1.77±0.13^a	1.55±0.18^b	1.41±0.10^b	1.44±0.15^b
Feed: Egg ratio	2.31±0.04^a	2.23±0.03^b	2.18±0.03^c	2.29±0.02^a

In the same row values with different small letter superscripts mean significant difference($P<0.05$), While with the same or no letter superscripts mean no significant difference($P>0.05$), The same as below.

Control group: fed basic diet (Table I). Group I, II and III were fed basic diet supplemented with 0.5%, 1% and 1.5% of CHM for 10 days and then fed with elemental diets for 7 days and again with CHM for 27 days.

Table IV. Effect of CHM additives on egg quality.

Items	Control group	Group I	Group II	Group III
Egg weight (g)	59.60±5.27[a]	60.19±4.50[a]	61.34±6.12[a]	59.61±3.60[a]
Eggshell weight (g)	6.37±0.62[a]	6.64±0.62[a]	6.71±0.49[a]	6.62±0.63[a]
Egg shape index	1.35±0.037[a]	1.35±0.062[a]	1.40±0.045[a]	1.34±0.046[a]
Yolk index	0.36±0.055[a]	0.32±0.044[a]	0.37±0.034[a]	0.36±0.033[a]
yolk color	4.40±0.84[b]	6.00±1.05[a]	6.00±0.82[a]	5.90±0.87[a]
Yolk viscosity	14.57±4.63[b]	20.42±5.36[a]	25.81±6.29[a]	25.40±5.28[a]

For details of groups see Table III.

Table V. Effect of CHM additives on common nutrition component of egg.

Items	Control group	Group I	Group II	Group III
Moisture (%)	34.18±2.15[a]	32.51±2.10[a]	30.07±2.79[a]	30.40±1.35[a]
Crude protein (%)	15.33±2.52[a]	14.67±2.51[a]	15.30±2.00[a]	15.00±1.73[a]
Crude fat (%)	43.73±0.38[b]	46.43±0.91[a]	46.87±0.36[a]	46.83±0.48[a]
Crude ash (%)	6.47±0.005[c]	6.20±0.004[d]	7.43±0.01[b]	7.53±0.01[a]
Calcium (%)	0.05±0.001[ab]	0.05±0.01[b]	0.06±0.00[a]	0.05±0.001[b]
Phosphorus (%)	0.25±0.005[a]	0.24±0.02[a]	0.26±0.01[a]	0.20±0.001[b]

For details of groups see Table III.

Effect of CHM additives on egg quality

Our CHM additives significantly increased the yolk color scores and viscosity was increased by 40.15, 77.18 and 74.33 % for groups I, II and III vs controls, respectively ($P < 0.05$). Both the egg and eggshell weights were also increased for the test groups but did not significantly differ from controls. Egg shape and yolk indices also were similar to the control values. Overall, group II performance scores exceeded those of groups I and group III but these differences were not significant ($P > 0.05$) (Table IV).

Effects of CHM additives on yolk nutritional components

The measurements of nutritional content of yolks indicated that water and crude protein levels decreased for the 3 test groups compared with controls but not by significant amounts ($P > 0.05$). However, crude fat content increased significantly by 6.17, 7.18 and 7.09 %, respectively ($P < 0.05$). The crude ash content in groups II and III increased significantly by 14.84 and 16.38% compared with controls, respectively ($P < 0.05$). Interestingly, the crude ash content in group I decreased significantly by 4.17 compared with controls ($P < 0.05$). The Ca content in group II was significantly higher than groups I and III ($P < 0.05$). In contrast, P content in group III was 16.67 and 23.07 % lower than for groups I and II and reached the level of statistical significance ($P < 0.05$) (Table V).

The amino acid detection chromatogram showed in Figure 1. Essential amino acid (EAA), non-essential

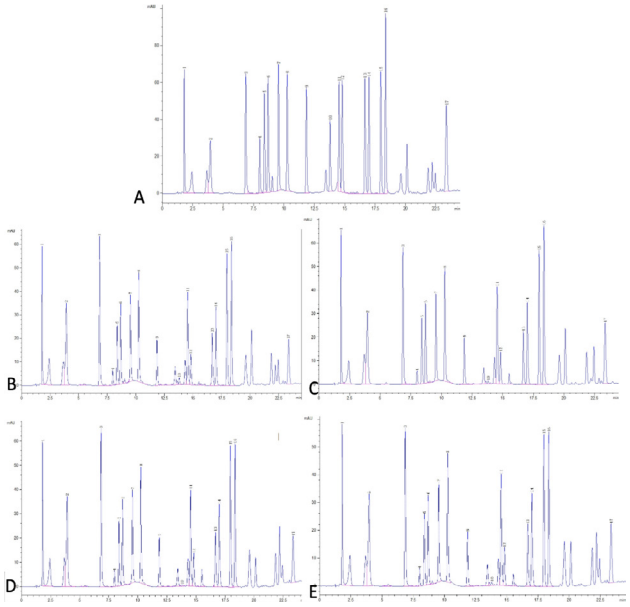

Fig. 1. Amino acids in eggs yolk by HPLC.
A, amino acid standards; B, Control group; C, Group I; D Group II; E Group III.
1, aspartic (asp); 2, glutamic (glu); 3, serine(ser); 4, histidine (his); 5, glycine (gly); 6, threonine (thr); 7, arginine (arg); 8, alanine (ala); 9, tyrosine (tyr); 10, cysteine (cys); 11, valine (val); 12, methionine (met); 13, phenylalanine (phe); 14, isoleucine (ile); 15, leucine (leu); 16, lysine (lys); 17, proline (pro).

Table VI. Effect of CHM additives on Amino acid content of egg yolk mg/g.

Amino acid	Control group	Group I	Group II	Group III
Lysine (Lys)*	10.63±0.22^{a}	9.73±0.88^{a}	10.49±1.05^{a}	9.52±0.19^{a}
Methionine (Met)*	3.54±0.15^{a}	3.12±0.26^{a}	3.64±0.12a	3.35±0.11^{a}
△Phenylalanine (Phe)*	6.76±0.12^{a}	5.77±0.46^{b}	6.62±0.17^{a}	6.18±0.15^{b}
Isoleucine (Ile)*	7.96±0.07^{a}	7.04±0.65^{b}	8.07±0.23^{a}	7.27±0.39^{b}
Leucine (Leu)*	12.66±0.42^{a}	11.01±0.84^{b}	12.27±0.36^{a}	11.64±0.36^{b}
Valine (Val)*	8.67±0.30^{a}	7.89±0.60^{a}	8.76±0.23^{a}	7.92±0.31^{a}
Threonine (Thr)*	7.41±0.18^{a}	6.35±0.47^{c}	7.06±0.24^{b}	6.82±0.24^{b}
△Aspartic (Asp)	13.41±0.39^{a}	12.02±1.08^{b}	13.81±0.58^{a}	12.29±0.35^{b}
△Glutamic acid (Glu)	20.28±0.52^{a}	16.43±1.20^{b}	18.86±0.78^{a}	18.57±1.62^{a}
Serine (Ser)	11.85±0.32^{a}	9.24±0.71^{c}	10.66±0.36^{b}	10.79±0.45^{b}
Histidine (His)	3.70±0.18^{a}	2.88±0.23^{b}	3.51±0.17^{a}	3.29±0.22^{a}
△Glycine (Gly)	4.07±0.15^{a}	3.76±0.29^{a}	4.06±0.14^{a}	3.80±0.12^{a}
Arginine (Arg)	10.85±0.32^{a}	9.13±0.70^{b}	10.39±0.31^{a}	9.92±0.40^{a}
△Alanine (Ala)	7.45±0.27^{a}	6.56±0.52^{b}	7.27±0.20^{a}	6.84±0.22^{b}
△Tyrosine (Tyr)	7.11±0.23^{a}	6.12±0.46^{b}	6.96±0.20^{a}	6.50±0.33^{b}
Cysteine (Cys)	0.68±0.48^{a}	0.52±0.12^{b}	0.41±0.04^{b}	0.66±0.46^{a}
Proline (Pro)	5.30±0.30^{b}	6.03±0.62^{a}	6.74±0.72^{a}	4.99±0.66^{b}
Essential amino acid (EAA)	57.63±1.44^{a}	50.91±4.10^{b}	56.91±2.00^{a}	52.70±1.37^{b}
Non-essential amino acid (NEAA)	84.69±3.13^{a}	72.69±5.65^{c}	82.67±2.36^{a}	77.80±2.50^{b}
Total amino acid (TAA)	142.32±4.56^{a}	123.59±9.74^{c}	139.58±4.31^{a}	130.49±3.86^{b}

Note: * denotes essential amino acids, △ denotes flavor amino acids. For details of groups see Table III.

amino acid (NEAA) and total amino acid (TAA) content for all 3 test groups were lower than controls. EAA, NEAA and TAA in test groups I and group III decreased by 11.66, 14.17, 13.16 and 8.55, 8.13, 8.31%, respectively. Compared with the control group, the EAAs methionine, isoleucine and valine and the NEAAs aspartic acid and proline increased significantly in group II and proline content increased by 27.17% compared controls ($P < 0.05$). TCAAs excluding proline for groups I and III were lower than controls and phenylalanine, isoleucine, leucine, threonine, aspartic acid, serine, and alanine were significantly lower than controls ($P < 0.05$). TCAA levels were the highest in group II except for serine and cysteine compared with both control and groups I and III (Table VI).

DISCUSSION

The use of Chinese herbal feed additives in poultry production is increasing and numerous experimental studies that indicated this improved poultry production. For instance, the addition of 0.25 and 0.50% *Ligustrum lucidum* (broad-leaved privet) to laying hen diets decreased mortality (Li *et al*., 2017). Aged Hyland brown hens (440 days old) fed a CHM containing *R. astragali, Radix codonopsis*, *Atractylodes* spp. (Daisy family), *Glycyrrhiza* (licorice), *Radix angelicae sinensis* (Danjjui), *Pericarpium citri reticulate* (Chenpi) and hemp seed significantly reduced mortality, broken (soft) egg levels and feed: egg ratios (Li *et al*., 2022; Liu, 2017). Our results were similar and our extracts increased egg numbers per animal, lowered egg breakage and feed: egg ratios. Overall, CHM additives promoted growth and development of laying hens, improved the utilization of protein, Ca and P, increased laying rates and reduced average feed consumption.

Egg quality is dependent of the feeding method, hen breed and age as well as feed nutrient levels (Zhang *et al*., 2019). The addition of 200 mg kg^{-1} rosemary essential oil to hen feed increased egg specific gravity, shell pore numbers and yolk color scores and protein content (Gaecia *et al*., 2019). These types of additives have also been demonstrated to increase eggshell color, and yolk protein content (Tang *et al*., 2020). The color of the egg yolk is primarily due to lutein disposition and can be enhanced by marigold supplements which are rich in lutein and lutein

esters. Our CHM additive also increased egg crude fat disposition, decreased yolk water content and increased yolk viscosity similar to other reports (Han, 2019).

The levels of the CHM mixture that we added to the feed was closely related to the prescription and dosage of traditional Chinese medicine additives. For example, *A. sinensis*, *Ligusticum chuanxiong*, *Rehmannia, Radix paeoniae rubra* and *Radix astragali* improved the color and relative weight of the egg yolk and increased shell thickness. The latter was positively correlated with the number of additives (Kowalska *et al*., 2021). Our results indicated an optimal at 1% additive and levels 50% higher or lower lessened the effects. Further experiments are required to identify the reasons for this behavior. However, dosage effects have been commonly seen for numerous types of Chinese herbal additives and have been found to alter yolk cholesterol levels and water content (Li *et al*., 2011). CHM extracts have also been given to laying hens in drinking water and water, crude protein, fat and ash in eggs decreased after adding peppermint and Tong's old stork extract (Dilawar *et al*., 2021).

Egg fat content affects flavor and higher levels result in a stronger flavor. We found that 1% CHM additive increased the levels of proline and aspartic acid that are key flavor enhancers. These eggs were found to be more flavorful using volunteer taste tests. Amino acid content also reflects the nutritional value of egg yolk and TAA, EAA and NEAA were most significant between test group I and the control group.

CONCLUSIONS

To conclude CHM additive to the diet of caged laying hens improved laying performance, egg quality and yolk nutritional content and significantly reduced egg breakage and the feed: egg ratio. This CHM also substantially increased laying rate, yolk color, viscosity and crude fat content and these positive effects were achieved at 1% addition to the standard hen diet.

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Ethical statement

Samples were obtained after due perission from the relevant institutions. All procedures were performed under the instructions and approval of the Laboratory Animals Research Centre of Henan province in China and the Ethics Committee of the Xinyang Agriculture and Forestry University

Statement of conflict of interest

The authors have declared no conflict of interest.

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