Effects of Astragalus, Epimedium, and Fructus Ligustri Lucidi extractive on antioxidant capacity, production performance, and immune mechanism of breeding pigeons under stress

Xue Zhang ,[*,1] Shuo Zhou ,[*,1] Yayan Liang,[*] Gaijie Xie,[*] Mingqiang Zhu,[*] Zifan Wang,[*] Qing Qu,[*] Yifei Long,[*] Yantao Lv,[*] Jie Peng,[*] Yaohui Yuan,[‡] Yanhua Huang,[*,†] and Wei Wang [*,†,2]

[*]*College of Animal Science and Technology, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong 510225, China;* [†]*Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou, Guangdong 510640, China; and* [‡]*Shenyang Weijia Biotechnology Co., Ltd, Shenyang 110027, Shenyang, Liaoning, China*

ABSTRACT With the large-scale and intensive development of pigeon breeding industry and the improvement of production level, stress factors have an important impact on the immune, antioxidant capacity, and productivity of pigeons. In this study, the extenuating effect of Astragalus, Epimedium, and Ligustrum lucidum (**AEF**) on the antioxidant, production performance, and immune mechanism was investigated in breeding pigeons. Eighty pairs of 11-month-old healthy breeding pigeons with the same egg production batch were randomly divided into 4 groups: control group (**C group**), treated with AEF (**AEF group**), in restraint stress (**S group**) and treated with AEF and in restraint stress (**S+AEF group**). Results showed that AEF reduces weight loss during lactation and increases spleen weight, increased IgA, IgG, T4, GSH-Px, and SOD in serum and decreased T3 and MDA ($P < 0.05$). Furthermore, treatment with AEF declined *HSP60, HSP70, HSP90, GR* levels in liver and *cFOS, GR* mRNA levels in the Hypothalamus, *GR* mRNA levels in the pituitary ($P < 0.05$). Meanwhile, the results of the intestine studies showed that AEF promoted relative abundances of *Firmicutes* and relieve intestinal injury in the colon of pigeons. These results indicated AEF enhanced stress resistance, immunity, production performance and antioxidant capacity of pigeons.

Key words: breeding pigeon, AEF, stress, immune performance, antioxidant capacity

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INTRODUCTION

Stress factors have a significant impact on the health and antioxidant capacity of pigeons due to the large-scale and intensive development of the pigeon breeding industry as well as the enhancement of production level (Xu et al., 2022a). Poultrys' production (egg laying rate, egg quality, fertilization rate, weight gain, etc.), health state and immunological biological index would all suffer greatly under heat stress, and various diseases will manifest. Stress causes the hypothalamus, pituitary gland, and adrenal cortical axis (**HPA axis**) overactive, which lowers reproductive effectiveness (Brix et al., 2022). Stress and the decline of egg laying ability are also important factors restricting the development of breading industry. It is increasingly urgent to find safe and effective drugs to inhibit the over activation of HPA axis and reduce the adverse impact of egg laying interval on breeding industry. Due to the overactivation of the HPA axis during the brood period, the immunity of pigeons decreases, which has an impact on the performance of suckling pigeons in terms of production, causing young pigeons to grow slowly, breeding pigeons to lay eggs more infrequently, and in severe cases, even death (Beckford et al., 2020; Austin et al., 2021; Xu et al., 2022a).

Stress also impair intestinal microbial balance (Hou et al., 2021). The intestinal microbiota contributes to poultry health through maintaining intestinal homeostasis, enhancing mucosal maturation, promoting immune system development, and inhibiting colonization of intestinal pathogens (Maynard et al., 2012; Wen et al., 2022). Balanced intestinal microbiota contributes to improving animal health through inhibiting pathogens, maintaining intestinal barrier integrity, promoting digestion and absorption (Abdelqader et al., 2020). The integrity and microbial balance of the gut, as well as any subsequent

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[1]These authors contributed equally to this work.

[2]Corresponding author: wang_wei99@jlu.edu.cn

effects on health, are heavily dependent on the early formation of the gut microbiota of newborn pigeons (Ding et al., 2021). The digestive system of newly hatched pigeons is sterile, immature and very sensitive to infection, which significantly affect nutrient utilization, feed efficiency, growth, immune response, and health status (Wen et al., 2022). Meanwhile, various harmful bacteria that increase hatched pigeons' susceptibility to diseases and could result in significant losses in poultry production (Jahanian and Rasouli, 2016). Using antibiotics to control diseases is a method often adopted by farms (Lianou and Fthenakis, 2022). Plant extracts is a feasible option to partially replace the antibiotics at the moment, as the need for an alternative antibiotic has become critical (Millar et al., 2021). Although the currently available medications and reproductive hormones have some impact, it might be challenging to prevent toxic side effects, drug residues, and toxic doses. Other frequently used antistress medications are easily oxidized and rendered ineffective during the adding process and it is challenging to determine the precise final therapeutic amount. Recent findings have focused a lot of emphasis on how to improve the intestinal microbiota and gut health of young fowl using natural feed ingredients and plant extracts (Abdelli et al., 2021; Shehata et al., 2022)

Plant extracts has so garnered a lot of interest because it is environmentally friendly, safe, and possesses the traits of having no drug residue, minimal adverse effects, and positive results, and it is particularly helpful at boosting immunity, increasing egg production, and preventing and treating stress. It is now a crucial method for reducing the negative effects of stress on animals both domestically and overseas (Liu and Yang, 2006). For the past several years, plant extracts has been increasingly used in poultry and mammals (Olawuwo et al., 2022).

Astragalus, Epimedium, and Ligustrum lucidum (**AEF**) were able to boost the host immunity and increase the growth performance in animals. However, there has been little research about the effect of AEF in drinking water on stress resistance, pigeons' egg production, immunity, and intestinal health. Therefore, we evaluated the effects of AEF on growth performance in young pigeon, immune function and intestinal microbiota in Parent pigeons, and potentially provided the theoretical basis for the application of AEF to improve production performance, antioxidant capacity, and immunity for pigeons.

Astragalus membranaceus, as green feed additives, are used as live microbial feed supplements to improve intestinal barrier function, microbial balance and gut health (Qiao et al., 2022). Astragalus and Epimedium have the potential to enhance pigs' development performance, feed efficiency, and immune responses by preserving the intestinal homeostasis (Jin et al., 2021). Ligustrum lucidum can be used as an effective feed additive to improve the performance of laying hens during the late laying period (Li et al., 2017). However, there has been little research about the effect of AEF in drinking water on stress resistance, pigeons' egg production, immunity, and intestinal health. Therefore, we evaluated the effects of AEF on growth performance in young pigeon, immune function and intestinal microbiota in Parent pigeons, and potentially provided the theoretical basis for the application of AEF to improve performance and immunity for pigeons. In view of the importance of production performance and the function of AEF, we assumed that AEF may enhanced stress resistance and immunity through inhibiting hypothalamic-pituitary-adrenal axis. Therefore, the present experiment was conducted to explore the effect of AEF supplementation on production performance by evaluating changes immunity and antioxidative capacity.

MATERIALS AND METHODS

Animal

A total of 80 pairs (40 males and 40 females,) of aged 11-month-old (French white pigeon × Deep king pigeon) breeding pigeon (*Columba livia*) with a similar performance from a commercial pigeon farm (Houde Meat Pigeon Industrial Development Co., Ltd, Guangzhou, China) were kept in a pathogen-free environment and fed ad lib. Eighty pairs breeding pigeon were randomly divided into 4 group (20 / group): control group (C Group), Add Astragalus, Epimedium, and Ligustrum lucidum extract (AEF) to drinking water group (AEF group), stress group (S group) and stress with AEF group (S+AEF group) (Table 1). The squab born from the test parent pigeons were also treated the same as the parent pigeons (Table 2). Set two cameras to record the behavior of breeding pigeons.

Plant Extracts

Take 100 g of Astragalus membranaceus (*ASTRAGALI RADIX*), 60 g of Epimedium (*EPIMEDII FOLIUM*), 60 g of Ligustrum lucidum (*LIGUSTRI LUCIDI FRUCTUS*), 40g of silver bupleurum (*STELLARIAE RADIX*), 40 g of Poria cocos (*PORIA*), and 30 g of licorice (*GLYCYRRHIZAE RADIX ET RHIZOMA*) and decoct the above drugs in 10 times, 8 times, and 5 times water for 3 times, each time for 30 min. Combine the decoction, filter, concentrate the filtrate to about 330 mL, add 3 times of ethanol, and let it stand at 4℃ for 20 h. Filter, recover ethanol from the filtrate, and concentrate it to a clear paste with a relative density of 1.25 to 1.35 (25℃). Add 3 portions of sucrose and 1 portion of dextrin according to the amount of clear cream, mix well, granulate, dry at low temperature, and then

Table 1. Pigeon breeding test grouping and scheme design.

Item	Add category	Give stimulation
C	normal water	noting
AEF	water with AEF	noting
S	normal water	restraint stress
S+AEF	water with AEF	restraint stress

Table 2. Experimental grouping and scheme design of squab.

Item	Give stimulation
c	noting
aef	noting
s	restraint stress
s+aef	restraint stress

pack separately. The concentration of Chinese medicine in water is 0.1 g/mL, and each pigeon is fed 5 mL once, twice a day.

Administration of AEF in the Pigeons and Tissue Collection

The experiment lasted for 43 d, including 18 d incubation period (pregnant) and 21 d lactation period (breed). AEF was dissolved in sterile normal saline for 0.1 g / mL and pigeons were treated with AEF for 43 d. Restraint stress was given in the last 5 d (not move for 20 min / day). Pigeons had ad libitum access to feed and water.

On the 43rd d, blood samples were collected into centrifugal tubes from the wing veins of pigeons in each group (n = 20) for serum preparation. After that, 20 pair pigeons in each group were sacrificed post-anesthesia and intestine were isolated and washed in cold PBS. Intestine, hypothalamus, liver and spleen tissues were used for determinations of quantitative real time polymerase chain reaction (**RT-qPCR**) or fixed in 4% neutral paraformaldehyde solution for morphological observation with H&E staining.

Determination of Serum

Blood samples were collected into centrifugal tubes from the wing veins of pigeons in each group. Determination of serum were TP, ALB, GLB, AST, ALT, TG, TC, UREA, P, and Ca carried out with automatic biochemical analyzer BS-240VET (Mindray Bio, Shenzhen, China). Detection of ACTH, CRF, CORT, T3, T4, PRL IgA, and IgG in pigeon serum. Elisa kits were used (Jiangsu Meimian industrial Co., Ltd, China). Detection of ACTH, CRF, CORT, T3, T4, PRL IgA, and IgG in pigeon serum. Specific steps were performed following instructions from the manufacturer. Approximately 50 μL aliquot of serum was used for the metabolome assay.

Morphological Observation

All tissues were fixed with 4% paraformaldehyde for 24 h at 4℃ and then processed and embedded in paraffin. Images of the slides were observed using Leica microscope (DM500, Leica, Wetzlar, Germany). The villus height (**Vh**) and crypt depth (**Cd**) were measured by Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD).

DNA Extraction and Sequencing

Feces samples from each group of four pigeons were collected for nucleic acid extraction. Total DNA was extracted using the RNeasy Power Microbiome KIT (Qiagen, Milan, Italy), following the manufacturer's instructions. The 16S rRNA gene V3−V4 region was amplified by gDNA to evaluate the microbiota (Klindworth et al., 2013). PCR products were tested on the Illumina MiSeq platform (Majorbio, Shanghai, China), following the instructions (Caporaso et al., 2012).

RT-qPCR Analysis

Total RNA was extracted from small intestine, liver, spleen, hypothalamus, pituitary using a Trizol reagent (Invitrogen, Carlsbad, CA). RNA concentrations and quality were measured using microspectrophotometer Q3000 (Quawell Technology, Inc., Sunnyvale, CA). and cDNA was generated using a commercial RT-PCR kit (Takara Shuzo Co., Ltd., Kyoto, Japan). The qPCR conditions were as follows: 95℃ for 30 s; 40 cycles of 95℃ for 5 s, 60℃ for 34 s; 95℃ for 15 s; 65℃ for 5 s; 95℃ for 5 s. Then, real-time PCR was conducted using the SYBR Green QuantiTect RT-PCR kit (Roche, South San Francisco, CA), and each sample was analyzed in triplicate. Primers were listed in Table 3.

Determination of Antioxidant Capacity

The activities of superoxide dismutase (**SOD**) and glutathione peroxidase (**GSH-Px**) and the content of malondialdehyde (**MDA**) in serum were measured according to the instructions of the kit (Nanjing Jiancheng Bioengineering Institute, China).

Treatment of Young Pigeons

During the growth performance test, fasting pigeons were weighed at the age of 1, 14, and 21 d and calculated the average daily gain, average daily feed intake, and feed to meat ratio after the test. The spleen, bursa of Fabricius, and thymus were weighed. The chest muscles with similar size, shape, and position were collected.

Slaughter Performance Determination

After weighing, squabs were sacrificed after anesthesia and pulled out the feathers by wet pulling method. The carcass weight, semi clean chamber weight, full clean chamber weight, and abdominal fat weight were examined electronic balance (ics-6102c, Shenzhen Anheng weighing instrument Co., Ltd., China), referring to NY/T 823-2020 terminology and measurement calculation method of poultry production performance (China, 2020). The slaughter rate, semi clean chamber rate, full clean chamber rate and abdominal fat rate were calculated.

Table 3. Gene-special primers used in the real-time quantitative reverse-transcription PCR.

Gene	Primer(5'→3')	Length(bp)
β-Actin	Forwar: GTGGATCAGCAAGCAGGAGT Reverse: TCATCACAAGGGTGTGGGTG	101
GR	Forwar:GCAAGCCTCCTATTTTATCTGACAC Reverse:GCCCATGTTTTCTTGCTTTATGCC	152
c*FOS*	Forwar: GAGGAGCCTACCTTCACCCT Reverse: GAAAAGCAGCTCGTCGAAGG	111
IL-1β	Forwar: AAGTGCTTCGTGCTGGAGTC Reverse: ACGGTACAGAGCGATGTTGA	99
IL-10	Forwar:GCTCTGAACTGCTGGATGAA Reverse:CTGGTGAAGGGTGCTGGT	119
CAT	Forwar: GAGGAACCCTCAGACTCATTTG Reverse: CCATCAGGAATACCACGATCAC	117
GSH-Px	Forwar: AAATACAGGGGCTCGGTGTC Reverse: GGTTCTTGCTGCCCAAACTG	158
HSP60	Forwar:AGCCAAAGGGCAGAAATG Reverse:TACAGCAACAACCTGAAGACC	208
HSP70	Forwar:TCCTGATGAGGCTGTTGCTT Reverse: GTCTGGGTTTGTTTGGTGGG	189
HSP90	Forwar: TCCTGTCCTGGCTTTAGTTT Reverse:AGGTGGCATCTCCTCGGT	285
Claudin-3	Forwar:GGACGAGAGCACCAAAGCCAAG Reverse:CCAGGAGACGGGGATGAGGTTC	88
Claudin-4	Forwar: CGCCAAAGCCAAGGTCAT Reverse:ACCATCGGGTTGTAGAAGTCC	120

Muscle Quality Measurement

The pH value and meat color of muscle were measured for 45 min and 24 h (stored at 4℃) using pH meter (model 205, detu company, Lenzkirch, Germany) and color difference meter (nr20xe, sanenshi Technology Co., Ltd., China). The meat strain hydraulic tester (rh-1000, Runhu Instrument Co., Ltd., China) was used to measure the muscle water loss rate.

Eggshell Quality

Pigeon eggs (n = 12) were used to determine the conventional indexes of egg quality. Egg white weight, egg yolk weight, and egg shell weight were weighed by electronic balance. The longitudinal and transverse diameters of eggs were measured using electronic digital calipers. The thickness of eggshell was measured by electronic digital micrometer. The color of eggshell was measured by 3 NH spectrophotometer (ns800, China).

Laying Performance

During the experiment, the total egg weight and laying date of breeding pigeons were recorded in the unit of repetition every day. The average egg weight and laying interval were calculated.

Statistical Analysis

The table data were analyzed with one-way ANOVA using the IBM SPSS Statistics V25.0 software (SPSS Inc., Chicago, IL). Differences between groups were determined using Duncan's multiple range test. The data are expressed as means ± SD, and the differences between treatments were considered statistically significant when $P < 0.05$ and highly significant when $P < 0.01$. Quantitative PCR was triplicates, and the representative results were shown. Statistical analysis was done with the help of Student's t test or one-way analysis of variance (**ANOVA**), analyzed using GraphPad Prism 7.0 (GraphPad Software, San Diego, CA). The P-value < 0.05 was considered statistically significant.

RESULTS

Effects of AEF on Body Weight, Energy Metabolism, Antioxidant Capacity, and Immunity of Pigeons

As shown in Figure 1A, the weight loss was the largest in S group. Astragalus, Epimedium, and Ligustrum lucidum extract (AEF) alleviated the weight loss of breeding pigeons. Meanwhile, the weight loss of breeding pigeons was reduced after treatment with AEF alone. Biochemical parameters of pigeons were detected in serum. Results showed that the content of TP, ALB, GLB, ALP, A/G, P, and Ca in serum was decreased and TC, TG, ALT, CK, T-Bil-V, AST and CREA-S content in serum were increased in stress. While the metabolic capacity decline was alleviated after AEF treatment (Table 4). In addition, the antioxidant indexes in serum were detected by ELISA. Compared with control group, the content of SOD, CAT and GSH-Px was decreased in the pigeon serum in stress and the MDA content was increased in the pigeon serum in stress. AEF improved the antioxidant capacity of pigeons (Figure 1B). Furthermore, compared with control group, the serum IgA and IgG content of breeding pigeons was increased significantly after AEF treatment and decreased remarkably in stress. Then AEF relieved the serum immunoglobulin decline induced by stress (Figure 1C). Meanwhile, AEF alleviated the decrease of feeding, lie down and rest, lactation, comb feathers and stand still of pigeons caused by stress (Table 5).

Serum Metabolome Analysis of Pigeons After AEF Treatment

Metabolomics analysis was carried out on the serum of pigeons treated with/without AEF. The serum metabolites in the control group and the AEF-treated group were generally similar (Figures 2A and 2B). Total 9 differential metabolites were screened out in the AEF group, as compared with the C group. The results of volcano map showed that the levels of Alanine, Phenylalanine, Homovanillic, Acetylglycine, gamma-Glutamylalanine, Indole-3-carboxylic acid, Pyrrole-2-carboxylic acid, GLCA, Hydroxyphenyllactic acid was significantly increased after AEF treatment (Figure 2C). Two main differential metabolic pathways, glutathione metabolic pathway and glucose alanine cycle pathway, were screened (Figure 2D).

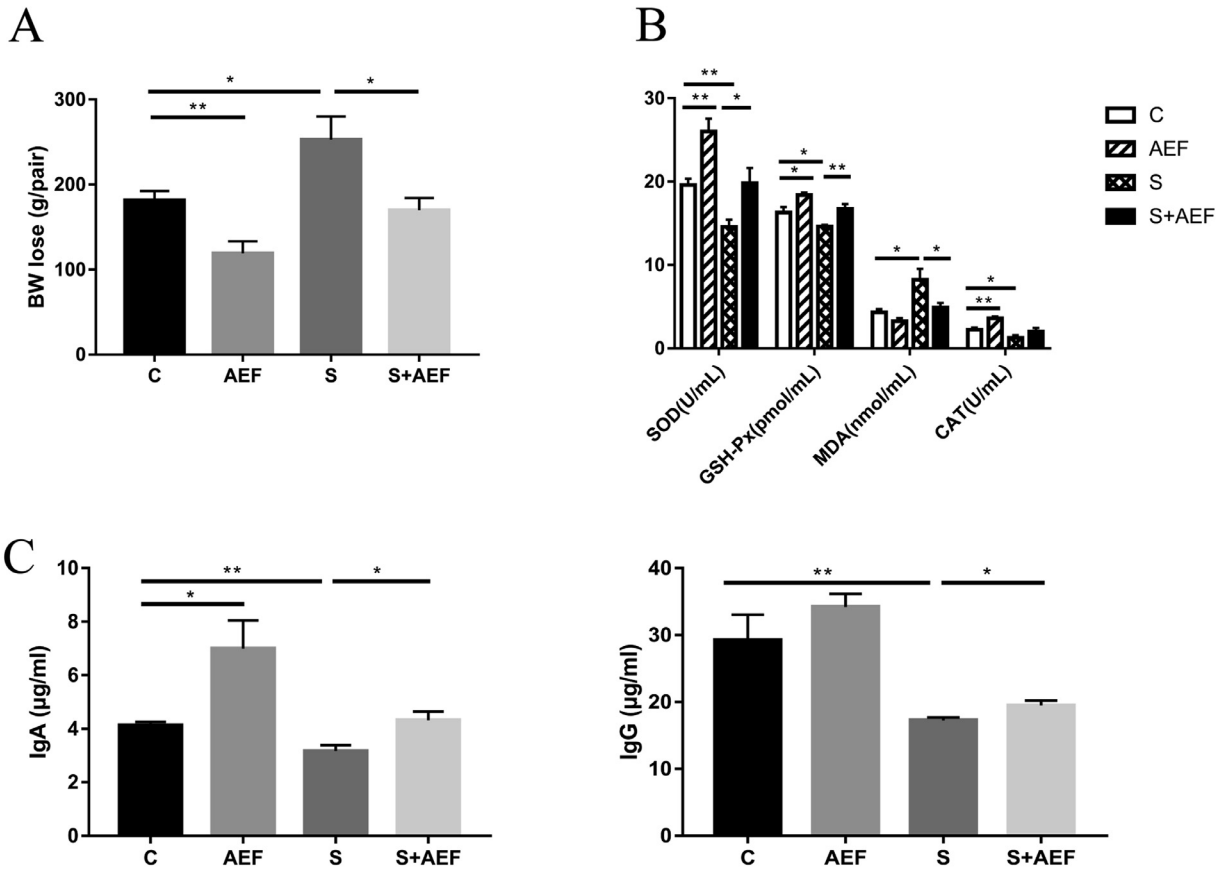

Figure 1. The antioxidant capacity and immunity changes of AEF on stress-induced pigeons. (A) Weight loss of lactating pigeons. Values are the means ± SEM (n = 6); (B) effects of AEF on serum antioxidant enzyme activity of pigeons. Values are the means ± SEM (n = 6); (C) immunoglobulin concentration in serum of pigeons. Values are the means ± SEM (n = 4-6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Effects of AEF on Hypothalamic-Pituitary-Adrenal Axis of Pigeons

As shown in Figure 3A, compared with control group, the content of ACTH, CRF, and CORT was remarkably increased in serum of breeding pigeons under stress. Treatment with AEF relieved the increasing ACTH, CRF, and CORT content effectively. However, blood glucose was significantly upregulated in breeding pigeons under stress and AEF could not reduce the rising trend of blood glucose. Meanwhile, The AEF improved the T_4 content and reduced the T_3 content of

Table 4. Effect of AEF supplements on serum biochemical parameters of pigeons.

Item	C	AEF	S	S+AEF	SEM	*P*-value
TP (mmol/L)	32.96 ± 3.94^{a}	33.76 ± 4.77^{a}	28.68 ± 3.93^{b}	32.86 ± 5.22^{a}	0.60	0.010
ALB (g/L)	11.51 ± 1.54^{A}	12.61 ± 1.96^{A}	8.61 ± 2.33^{C}	11.06 ± 2.00^{B}	0.30	<0.001
GLB (g/L)	20.81 ± 3.60^{B}	24.89 ± 6.32^{A}	17.23 ± 2.79^{C}	20.95 ± 4.05^{B}	0.64	<0.001
TC (mmol/L)	7.14 ± 0.91^{B}	6.63 ± 0.84^{B}	8.02 ± 1.42^{A}	6.69 ± 0.83^{B}	0.14	0.001
TG (mmol/L)	1.20 ± 0.60^{a}	0.94 ± 0.31^{a}	1.34 ± 0.95^{a}	0.73 ± 0.15^{b}	0.08	0.022
ALT (U/L)	29.60 ± 4.95^{B}	30.52 ± 6.05^{B}	44.07 ± 9.08^{A}	34.13 ± 8.42^{B}	1.15	<0.001
AST (U/L)	121.59 ± 15.85^{BC}	110.13 ± 16.54^{C}	139.72 ± 22.49^{A}	127.82 ± 20.29^{AB}	2.68	0.001
ALP (U/L)	289.56 ± 28.05^{AB}	299.64 ± 23.08^{A}	152.73 ± 17.61^{C}	280.54 ± 26.27^{B}	8.09	<0.001
A/G	0.57 ± 0.11^{A}	0.61 ± 0.11^{A}	0.45 ± 0.15^{B}	0.59 ± 0.08^{A}	0.02	0.001
P (mmol/L)	1.16 ± 0.42^{B}	1.59 ± 0.61^{A}	0.78 ± 0.32^{C}	1.16 ± 0.49^{B}	0.07	<0.001
Ca (mmol/L)	2.42 ± 0.70^{B}	3.17 ± 1.20^{A}	1.97 ± 0.52^{B}	2.55 ± 0.91^{B}	0.12	0.003
CREA-S (μmol/L)	15.69 ± 10.74^{B}	17.51 ± 7.82^{B}	44.43 ± 13.43^{A}	14.86 ± 5.64^{B}	1.97	<0.001
CK (U/L)	456.63 ± 18.01^{C}	440.99 ± 20.87^{C}	849.44 ± 29.50^{A}	676.76 ± 28.97^{B}	21.47	<0.001
TBA (μmol/L)	82.75 ± 6.60^{C}	76.48 ± 3.11^{D}	94.59 ± 3.25^{A}	85.98 ± 3.99^{B}	0.99	<0.001
T-Bil-V (μmol/L)	14.22 ± 3.96^{b}	15.75 ± 5.65^{b}	19.71 ± 7.05^{a}	15.01 ± 4.11^{b}	0.70	0.025

$^{ab;ABC}$In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), and different upper case letter superscripts mean extremely significant difference ($P < 0.01$). (n = 16; means ± SD). TP (Total protein), ALB (Albumin), GLB (Globulin), TC (Total cholesterol), TG (Triglyceride), ALT (alanine aminotransferase), AST (Aspartate aminotransferase), ALP (Alkaline phosphatase), A/G (the Albumin and Globulin ratio), P (Phosphorus), Ca (Calcium), CREA-S (Serum creatinine) CK (Creatine kinase) TBA (Total bile acid) and T-Bil-V (Total bilirubin).

Table 5. Effect of AEF on behavior of pigeons during lactation.

ITEM	C	AEF	S	S+AEF	SEM	*P*-value
Feeding	107.75 ± 5.65^{B}	123.63 ± 7.19^{A}	86.75 ± 7.09^{C}	103.88 ± 5.84^{B}	2.60	<0.001
Peck health sand	27.25 ± 3.33	27.88 ± 5.79	25.25 ± 4.17	26.00 ± 2.07	0.71	0.571
drinking water	23.63 ± 3.25	23.00 ± 3.59	25.63 ± 3.96	25.25 ± 3.58	0.64	0.415
lactation	55.63 ± 7.15^{B}	81.38 ± 8.90^{A}	35.75 ± 6.48^{C}	56.88 ± 11.91^{B}	3.27	<0.001
Lie down and rest	252.63 ± 22.18^{A}	239.50 ± 34.68^{A}	194.50 ± 32.28^{B}	228.13 ± 24.07^{A}	6.20	0.003
Comb feathers	248.88 ± 17.25^{AB}	266.25 ± 30.38^{A}	203.88 ± 34.82^{C}	226.75 ± 33.60^{BC}	6.55	0.002
stand still	231.88 ± 18.06^{B}	230.25 ± 16.50^{B}	292.63 ± 24.01^{A}	243.38 ± 21.00^{B}	5.67	<0.001
walk about	56.38 ± 14.13	55.13 ± 15.81	53.75 ± 14.55	54.13 ± 10.89	2.35	0.982
playing	15.13 ± 2.47	16.88 ± 3.14	16.38 ± 3.42	17.25 ± 3.11	0.53	0.539

^{ABC}In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), and different upper case letter superscripts mean extremely significant difference ($P < 0.01$). (n = 8; means ± SD).

breeding pigeons upon stress exposure (Figure 3B). The results of the RT-qPCR analysis showed that treatment of AEF reduced the expression of *cFOS* in the stress-treated in breeding pigeons compared with the S group (model control). The mRNA expression of *GR* had increased remarkably under stress in breeding pigeons compared with that of the control. However, these descending changes were all prevented by simultaneous AEF supplementation (Figure 3D). Next, the weight of spleen was measured. Stress induced spleen atrophy and it was inhibited by AEF treatment (Figure 3E). As shown in Figure 3F, the results show that the mRNA expression of *HSP60, HSP70* and *HSP90* was up-regulated in breeding pigeons after stress. AEF effectively alleviated the stress induced the increasing mRNA expression of *HSP60, HSP70*, and *HSP90*.

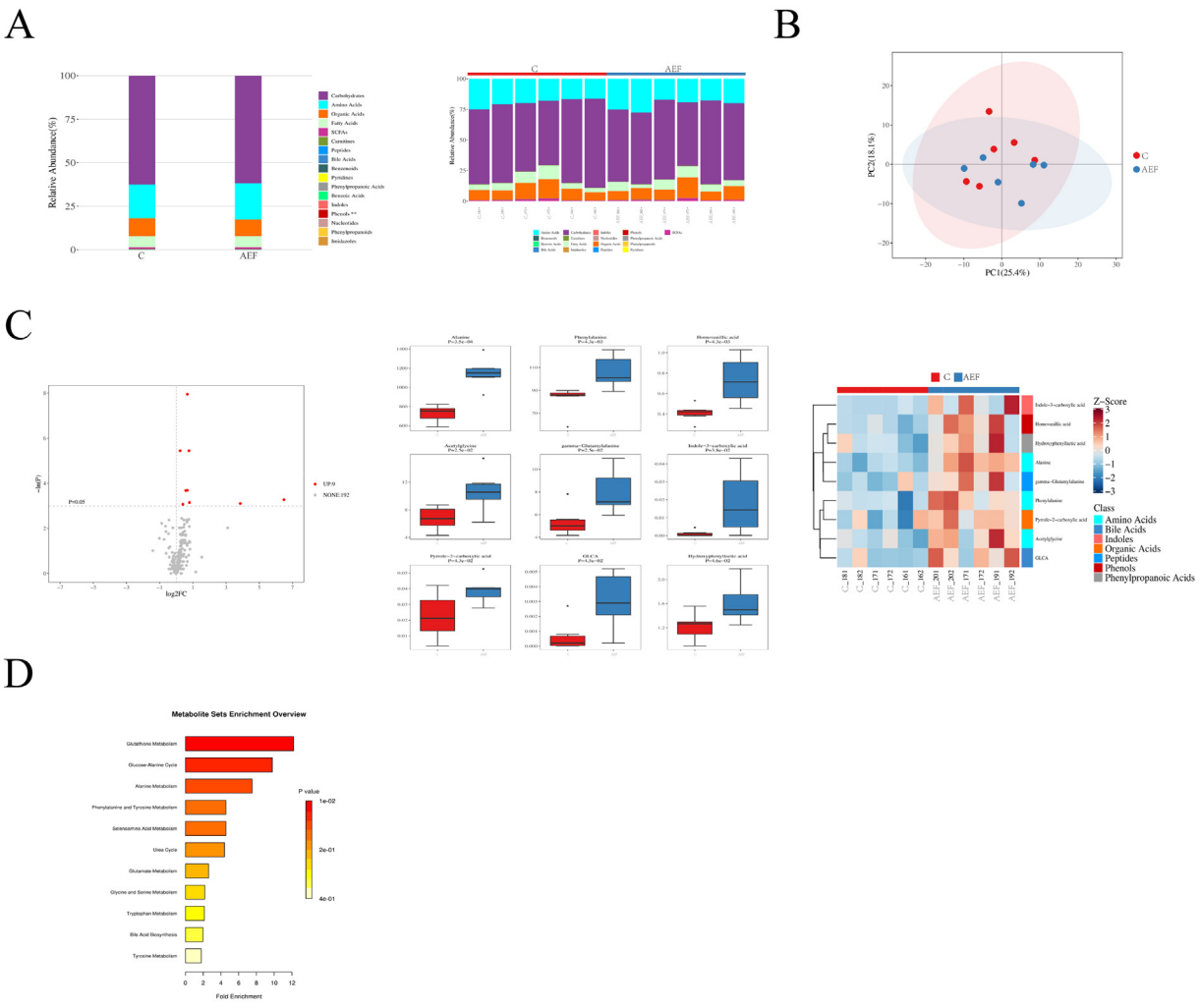

Figure 2. Metabolome analysis of pigeon serum. (A) Classification of metabolites; (B) PCA score diagram of serum samples; (C) one dimensional metabolite volcano map; (D) one dimensional differential metabolite box diagram; (E) potential biomarker heat map; (F) pathway-associated metabolite sets (SMPDB).

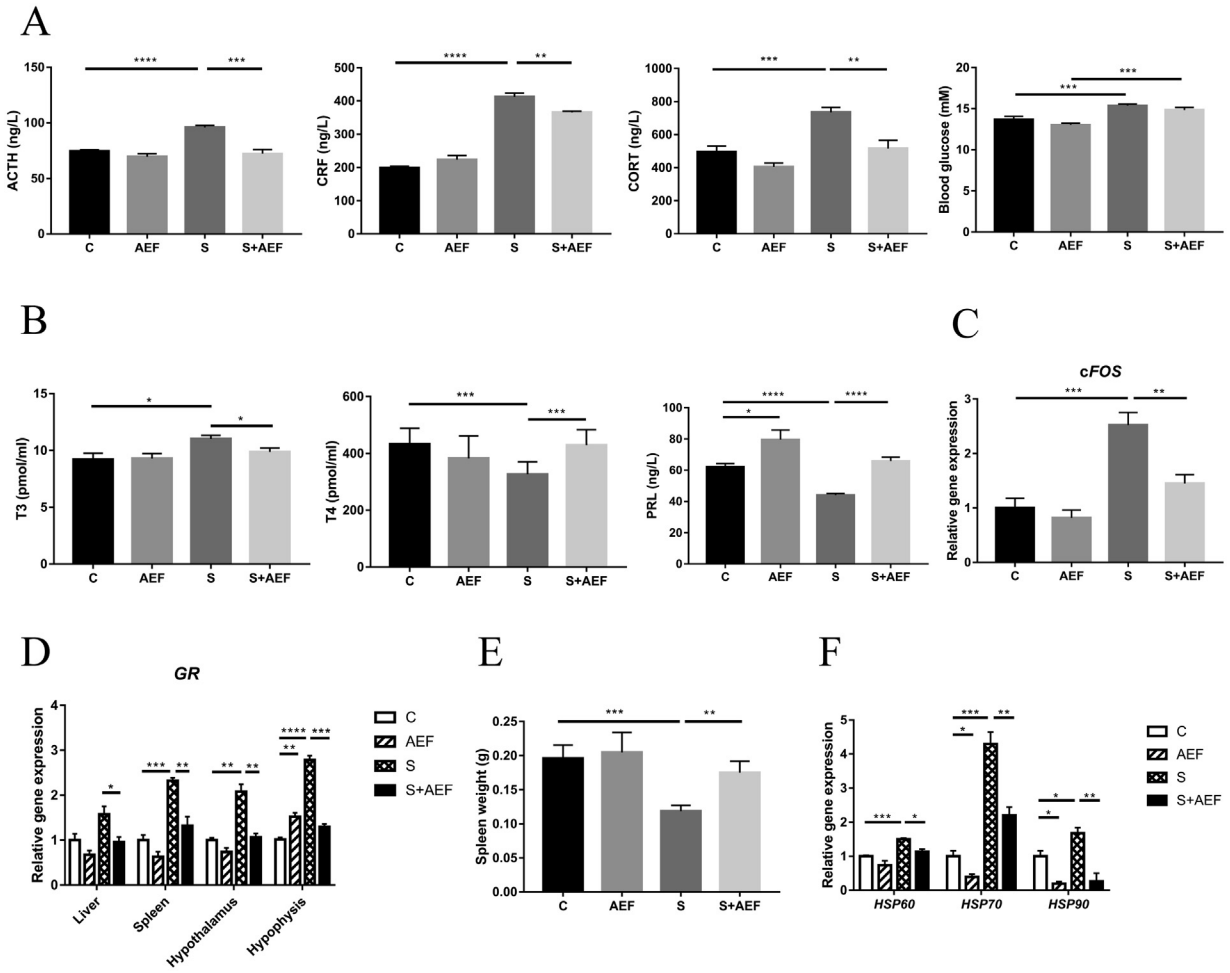

Figure 3. Effect of AEF on pigeon HPA axis. (A) The content of serum ACTH, CRF, CORT, and blood glucose in pigeon. Values are the means ± SEM (n = 6). $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (B) The content of T3, T4, and PRL in pigeon serum. Values are the means ± SEM (n = 3). $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$; (C) expression of *cFOS* gene in hypothalamus. Values are the means ± SEM (n = 3). $^{**}P < 0.01$, $^{***}P < 0.001$; (D) expression of *GR* mRNA in liver, spleen, hypothalamus, and hypophysis. Values are the means ± SEM (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; (E) weight of pigeon spleen (n = 30). $^{**}P < 0.01$, $^{***}P < 0.001$; (F) Expression of *HSP60, HSP70*, and *HSP90* mRNA in liver. Values are the means ± SEM (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Relieving Effect of AEF on Small Intestine Under Stress

The structure of duodenum, jejunum, and ileum in breeding pigeons under stress displayed sparse and shorter villi. The statistics of VH (villus height) and CD (depression depth) of duodenum, jejunum, and ileum showed that the VH / CD of small intestine was significantly downregulated under stress and AEF treatment effectively improved VH thus to restore the VH / CD ratio (Figure 4A). Several inflammatory factors mRNA expression were detected to evaluate the impact of feeding AEF on intestinal immune function. Compared with control group, the mRNA expression of *IL-1β* was enhanced in duodenum, jejunum, and ileum of breeding pigeons under stress. The mRNA expression of *IL-10* showed the opposite trend (Figure 4B). Meanwhile, the results of the RT-qPCR analysis showed that treatment of AEF reduced the expression of Claudin-3 and Claudin-4 in the stress-treated in breeding pigeons compared with the S group (model control) (Figure 4C). Finally, the expression of antioxidant factors in the intestine was evaluated. Compared with the group without AEF, the group with AEF significantly enhanced the antioxidant capacity (Figure 4D).

Enhanced Abundance of Colonic Contents Under Stress After AEF Treatment in Pigeons

The microbial composition of pigeon colon contents was detected by 16S rRNA Illumina MiSeq. In this study, 1,643,093 effective sequences were screened from 16 samples for subsequent analysis. Each sample in the content has an average of 851 operational taxons (**OTUs**). These 4 groups have obvious differences in phylum and family. The column chart of colony structure at phylum level showed that the relative abundance of Firmicutes in four groups were 61.94, 55.94, 32.17, and 36.26%, respectively. Meanwhile, AEF treatment decreased the increasing Proteobacteria in pigeon colon

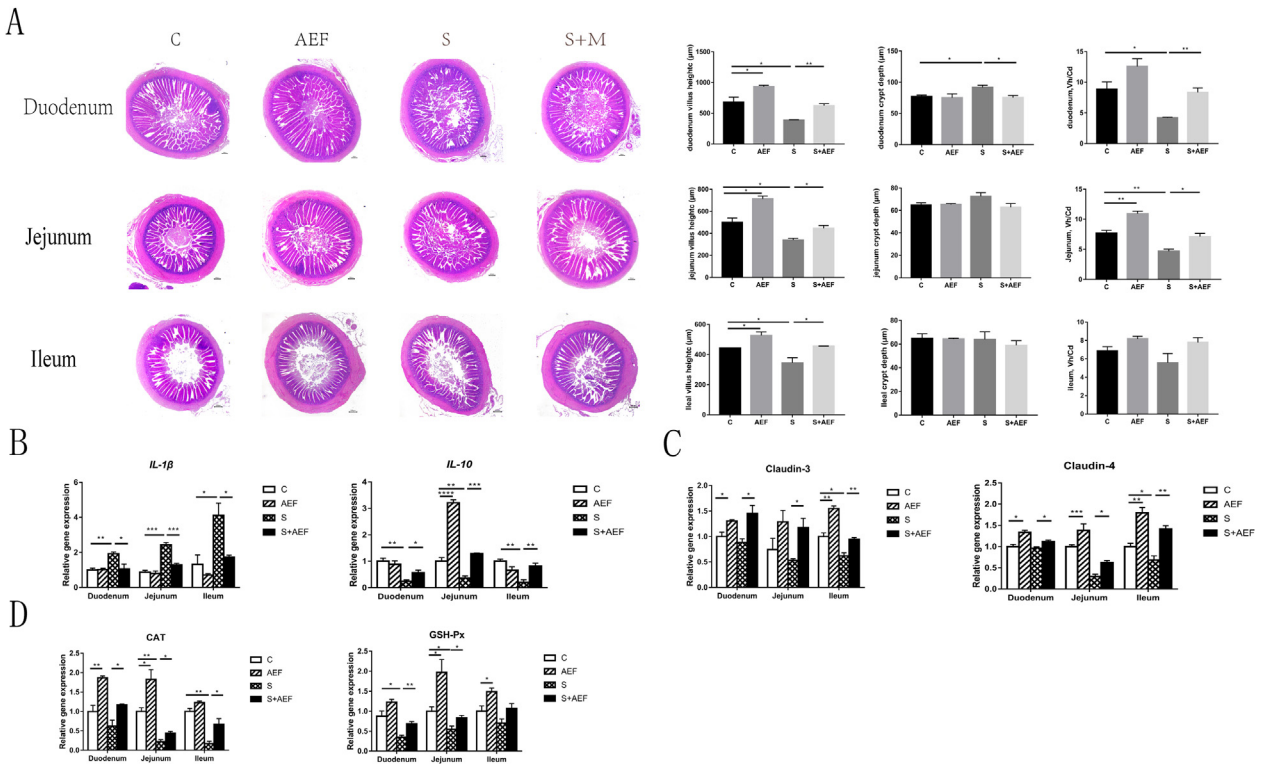

Figure 4. Changes of morphology and mRNA expression on small intestine. (A) Representative morphology and Vh/Cd of small intestine in pigeons. Scale bar: 200 μm. Values are the means ± SEM (n = 3). *$P < 0.05$, **$P < 0.01$; (B) the mRNA expression of *IL-1β* and *IL-10* in duodenum, jejunum and ileum by RT-qPCR. Values are the means ± SEM (n = 3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; (C) the mRNA expression of *Claidin-3* and *Claidin-4* in duodenum, jejunum, and ileum by RT-qPCR. Values are the means ± SEM (n = 3). *$P < 0.05$, **$P < 0.01$. (D) The mRNA expression of CAT and GSH-Px in duodenum, jejunum, and ileum by RT-qPCR. Values are the means ± SEM (n = 3). *$P < 0.05$, **$P < 0.01$. Abbreviations: Cd, crypt depth; Vh, villus height.

contents under stress (Figures 5A and 5B). The observed species count the number of visually observed species, Shannon's diversity index and Chao1 index reflex the richness and evenness of species, respectively. Compared the control group, the Chao1 index and observed species count showed a decreasing trend of pigeon under stress, indicating the decline of species types and richness. Treatment with AEF restored species richness to a certain extent (Figure 5C). Results showed that the sample sequencing volume is large enough to reflect the vast majority of microbial species information in the sample. Additionally, the sample rarefaction curve shows that with the increase in the number of sequencing samples, the 4 samples' OTU rarefaction curves tend to be smooth (Figure 5D). The Specaccum (species accumulation curve) showed the same result (Figure 5E). Meanwhile, the PCoA results with binary Jaccard distance verified that four groups were separated (Figure 5F). Species clustering tree showed that there were significant differences among different groups (Figures 5G and 5H). We obtain 5,389, 5,802, 1,193, and 1,241 OTU samples from C, AEF, S, and S+AEF group, respectively, with 263 shared OTU between the 4 groups (Figure 5I). The relative abundance of 16S rRNA gene sequence in the contents of pigeon colon is shown in, and the heat map depicts the most important 20 species of bacteria at genus level in the contents (Figure 5J). There is a certain degree of separation between the control group expanded with the blue marked points as the center and the other three groups (Figure 5K).

Effects of AEF on Squab

Pigeons of similar weight were selected for the experiment. Results showed that the stress group was the lowest in final body weight in 1 to 21 d. Compared with c group, the final body weight, ADG and ADFI in 1 to 21 d were decreased in s group. Meanwhile, the ADG of aef group was significantly higher than that of c group at 1 to 21 d. Furthermore, compared with s group, the ultimate weight, ADG, and ADFI of s+aef group were significantly increased. However, treatment with AEF alleviated the final body weight, ADG and ADFI decline induced by stress (Table 6). Compared with c group, the thymus index, bursa index, and spleen index of squabs under stress decreased significantly. Meanwhile, compared with s group (model control), the thymus index, bursa index, and spleen index after AEF treatment were significantly increased (Table 7). The slaughtering rate of AEF-treated squab was the highest among the 4 groups (Table 8). Compared with c group, the a*, b*, dripping loss and cooking loss increased significantly in squab under stress. Compared with s group (model control), the dripping loss and cooking loss of meat quality were significantly reduced after treatment with AEF (Table 9).

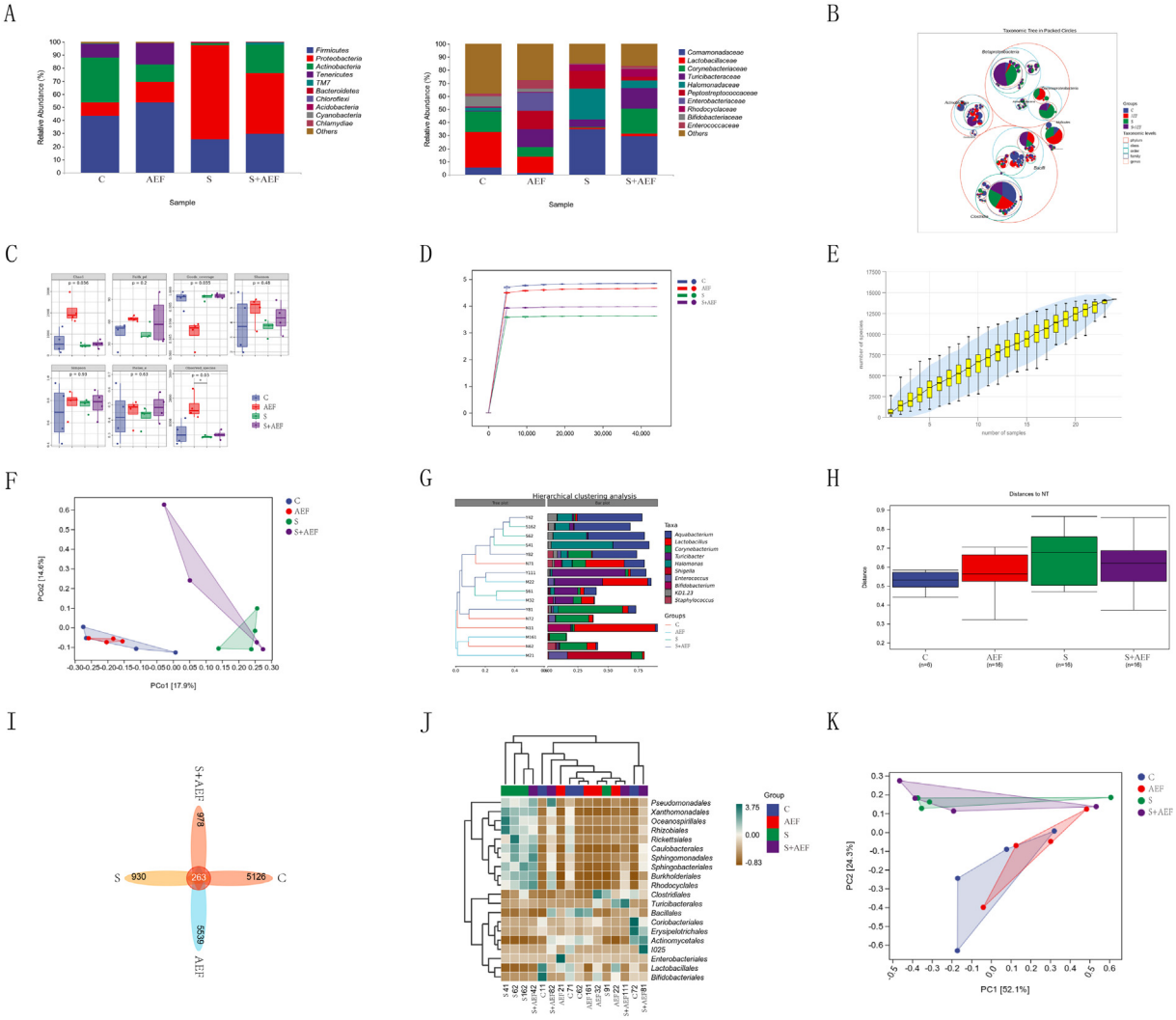

Figure 5. The abundance and diversity of colonic contents. (A) Phylum-level and Family-level relative abundance of 16S rRNA gene sequences from the colonic contents of pigeons (n = 4); (B) Taxonomic_tree; (C) Alpha diversity index; (D) Rarefaction curve based on Shannon index per group; (E) species accumulation curve; (F) principal coordinates analysis (PcoA) (based on the Binary-Jaccard); (G) hierarchical clustering analysis; (H) analysis of differences between groups; (I) petals figure; (J) species composition heat map; (K) PCA analysis diagram. Values are the means ± SEM (n = 4).

Changes on Egg Quality After AEF Treatment

In each group, 12 eggs for 7 consecutive days were randomly collected to assess egg quality parameters. After supplementation with AEF in the diet, egg weight, egg white weight, eggshell thickness, eggshell weight, and eggshell color were significantly increased. Egg laying interval was significantly reduced (Table 10).

Table 6. Effect of AEF supplements on growth performance of squab from 1 to 14 d and 1 to 21 d of age.

Item	c	aef	s	s+aef	SEM	*P*-value
Initial BW/g	22.40 ± 4.52	21.52 ± 4.13	22.64 ± 3.71	22.90 ± 3.34	0.56	0.843
Final BW/g	400.87 ± 8.37^{B}	411.66 ± 10.93^{A}	352.93 ± 8.91^{C}	398.03 ± 7.62^{B}	3.52	<0.001
1−14 d						
ADG/g	25.20 ± 2.47	24.92 ± 1.80	25.46 ± 2.74	26.68 ± 2.27	0.34	0.279
ADFI/g	115.21 ± 4.24	115.82 ± 5.09	115.82 ± 5.06	116.59 ± 5.33	0.70	0.925
F/G	2.26 ± 0.42	2.28 ± 0.65	2.05 ± 0.49	2.20 ± 0.41	0.07	0.683
1−21 d						
ADG/g	18.45 ± 1.30^{B}	20.40 ± 2.23^{A}	15.48 ± 1.79^{C}	18.82 ± 2.21^{AB}	0.37	<0.001
ADFI/g	121.13 ± 3.30^{A}	119.23 ± 4.15^{A}	107.60 ± 4.12^{B}	118.45 ± 4.98^{A}	1.00	<0.001
F/G	3.07 ± 0.43	2.96 ± 0.24	3.32 ± 0.56	3.26 ± 0.45	0.06	0.185

^{ABC}In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), and different upper case letter superscripts mean extremely significant difference ($P < 0.01$). (n = 12; means ± SD). Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; Final BW, final body weight; F/G, feed conversion ratio; Initial BW, Initial body weight.

Table 7. Effect of AEF on immune index of squab.

ITEM	c	aef	s	s+aef	SEM	*P*-value
Thymus index (%)	0.66 ± 0.18^{AB}	0.79 ± 0.14^{A}	0.42 ± 0.20^{C}	0.60 ± 0.16^{B}	0.03	<0.001
Bursa index of Fabricius (%)	0.21 ± 0.05^{B}	0.27 ± 0.08^{A}	0.14 ± 0.04^{C}	0.20 ± 0.04^{B}	0.01	<0.001
Spleen index (%)	0.14 ± 0.03^{B}	0.18 ± 0.06^{A}	0.09 ± 0.03^{C}	0.14 ± 0.03^{AB}	0.01	<0.001

[ABC]In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), and different upper case letter superscripts mean extremely significant difference ($P < 0.01$). (n = 12; means ± SD).

Table 8. Effect of AEF on slaughter performance of squab.

Item	c	aef	s	s+aef	SEM	*P*-value
Carass yield	89.68 ± 5.45^{ab}	91.33 ± 2.51^{b}	87.72 ± 3.23^{a}	88.20 ± 2.93^{a}	0.47	0.027
Semi-eviscerated yield	80.29 ± 3.70	82.88 ± 2.74	80.92 ± 2.79	79.96 ± 3.75	0.41	0.053
Eviscerated yield	63.37 ± 3.27	62.60 ± 6.83	60.59 ± 3.25	61.56 ± 4.73	0.58	0.351
abdominal fat	0.42 ± 0.23	0.34 ± 0.32	0.27 ± 0.16	0.29 ± 0.16	0.03	0.222

[ab]In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different lowercase letter superscripts mean significant difference ($P < 0.05$), and different letter superscripts mean extremely significant difference ($P < 0.01$). (n = 17; means ± SD).

Table 9. Effect of AEF on pigeon meat quality.

ITEM	c	aef	s	s+aef	SEM	*P*-value
Ph(0h)	6.25 ± 0.10	6.34 ± 0.17	6.41 ± 0.19	6.33 ± 0.18	0.02	0.051
Ph(24h)	5.85 ± 0.06	5.88 ± 0.12	5.83 ± 0.15	5.81 ± 0.10	0.01	0.367
L*	39.18 ± 2.47	40.63 ± 2.56	40.55 ± 2.46	40.02 ± 2.17	0.30	0.312
a*	11.20 ± 1.64^{B}	10.90 ± 1.24^{B}	13.49 ± 1.58^{A}	13.22 ± 1.34^{A}	0.23	<0.001
b*	9.98 ± 1.75^{B}	10.71 ± 1.11^{B}	12.23 ± 1.63^{A}	12.28 ± 1.92^{A}	0.23	<0.001
Drip loss	4.37 ± 1.59^{B}	4.71 ± 1.16^{B}	8.45 ± 1.66^{A}	4.16 ± 2.03^{B}	0.30	<0.001
Cooking loss	15.63 ± 2.19^{B}	14.45 ± 2.79^{B}	19.59 ± 4.82^{A}	15.08 ± 2.52^{B}	0.47	<0.001

[AB]In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), and different upper case letter superscripts mean extremely significant difference ($P < 0.01$). (n = 16; means ± SD).

Table 10. Effect of AEF on the quality of pigeon eggs.

ITEM	Egg weight (g)	Egg yolk weight (g)	Egg white weight (g)	Eggshell thickness (mm)	Eggshell weight (g)	Eggshell color	Egg shape index	Egg laying interval (day)
C	18.95 ± 1.28^{B}	4.37 ± 0.46	11.21 ± 1.32^{b}	0.22 ± 0.03^{B}	2.11 ± 0.10^{b}	82.46 ± 0.97^{B}	1.37 ± 0.02	34.67 ± 1.72^{a}
AEF	20.65 ± 1.53^{A}	4.60 ± 0.39	12.67 ± 1.34^{a}	0.25 ± 0.02^{A}	2.21 ± 0.10^{a}	84.43 ± 1.56^{A}	1.35 ± 0.04	32.75 ± 2.05^{b}
SEM	0.33	0.09	0.31	0.01	0.02	0.33	0.01	0.43
P-value	0.007	0.201	0.013	0.009	0.017	0.001	0.279	0.021

[ab,AB]In the same column, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different lowercase letter superscripts mean significant difference ($P < 0.05$), and different upper case letter superscripts mean extremely significant difference ($P < 0.01$). (n = 12; means ± SD).

DISCUSSION

The health and productivity of pigeons are significantly impacted by stress factors due to the extensive and intensive expansion of pigeon breeding and the enhancement of production level. Stress-exposed birds perform poorly in terms of growth (Sanchez-Casanova et al., 2022). Stressed birds appear to perform worse because they eat less and have less of an appetite, which is a strategy used by birds to lower metabolic heat generation. The use of natural products (e.g., Plant extracts) has been widely promoted in order to alleviate the negative effects of environmental pressure (Saleh et al., 2019; Albasher et al., 2020; Alagawany et al., 2022; Song, et al., 2022). Although there is evidence that Astragalus membranaceus reduces appetite and weight gain, it is unclear how it reduces stress. In this study, the effect of Astragalus, Epimedium, and Ligustrum lucidum extract (AEF) on immunity and antioxidant capacity of breeding pigeons was explored. These results showed that, supplementation in the water with 0.1 g / mL AEF resulted in enhanced anti-stress capacity. Therefore, addition of AEF in water could promote pigeon growth.

When the body is triggered by different internal and external environmental variables, stress is a systemic, nonspecific adaptive reaction that develops. Oxidative stress is characterized by an increase in lipid peroxidation and damage to cell function, a decrease in the body's antioxidant enzyme activity, and a decrease in the activity of antioxidant enzymes in pigeons. Pigeons that are lactating deposit more eggs, which lowers their resistance and increases the likelihood that they may induce oxidative stress (Wang et al., 2021a). CAT, GSH-PX, and MDA are significant antioxidant indicator activities that help identify the presence of antioxidants, which are essential for shielding cell structures from the damaging effects of reactive oxygen species caused by aging (Surai et al., 2019). The content of MDA (an important biomarker of oxidant status) directly reflected the level and degree of LPO and the body lipid damage caused by active free radical attack. Previous studies showed that the addition of Chinese herbal extracts such as anise extract, magnolol, and yucca extract to the diet improved the antioxidant status of pigeons (Sun et al., 2019; Faried and El-Mehi, 2020; Bibi et al., 2022). In this study, the effect of AEF on antioxidant status under stress of breeding pigeons was explored. The activities of some key antioxidant enzymes in serum were examined. Results showed that AEF supplemented with drinking water increase the levels of serum cat, SOD, GSH-PX, and reduce the level of MDA. In summary, AEF supplementation in water had an antioxidant effect and increased breeding pigeons' antioxidant capacity. These findings are in line with those of aquatic and terrestrial mammals (Wang et al., 2021b; Sonmez et al., 2022). The activity of SOD, GSH-PX and CAT in the serum of weaned pigs was improved by Astragalus membranaceus (Wu et al., 2021). Additionally, Ligustrum lucidum supplements reduced oxidative stress in various animal experiment trials (Liu et al., 2020). Epimedium decreased MDA and lipid peroxidation while increasing the enzyme activity of SOD, CAT, and GSH-PX in mice's serum and liver (Munir et al., 2020). In this study, AEF promoted the expression of CAT and GSH-PX in the small intestine of stressed pigeons, indicating that it enhanced the intestinal antioxidant stress.

Pigeons' immunity will decrease after stress and their immune system will be triggered and manufacture the necessary antibodies. In this study, AEF treatment has a greater concentration of IgA. Meanwhile, AEF effectively relieved the decrease of IgA and IgG caused by stress. These results showed that AEF treatment significantly improved the humoral immune response and the immune system performance of pigeons under stress. Inflammatory factors are one of the important indicators in the process of immune response. Stress impairs intestinal barrier function and triggers an inflammatory response (Tao et al., 2022). IL-1β caused acute or chronic inflammation. Addition of AEF down-regulated the proinflammatory cytokine IL-1β encoded in the small intestine of pigeons. At the same time, the expression of anti-inflammatory cytokine IL-10 was upregulated. In addition, AEF played a similar role in the duodenum, ileum, and jejunum of pigeons.

The stress response of poultry is mainly mediated by the activation of the hypothalamus pituitary adrenal (**HPA**) axis of the sympathetic nervous system (Kadhim et al., 2020). The main glucocorticoid or stress hormone in pigeons is cortisol. The increase of blood glucose may be part of the fight or flight response to help chickens survive. Birds living under stress have higher circulating glucose levels (Xie et al., 2015). This is also consistent with the results of this experiment. In addition to corticosterone, other hormones are also affected during stress. In addition to corticosterone, other hormones are also affected during stress (Beckford et al., 2020). For example, thyroid hormone and prolactin related to reproduction, stress will lead to hormone abnormalities, which can be regulated by AEF. Our results confirm that stress affects the adrenocorticotropic axis. In addition to the elevated levels of ACTH, CRF, and CORT in the circulation, stress also significantly reduced the *GR* mRNA levels in the hypothalamus and pituitary. Glucocorticoid is the most important regulator of stress response. When the body makes a "fight or flight" response, the secretion level of the stress hormone glucocorticoid will increase. Glucocorticoids can act on the hypothalamus and pituitary (inhibit the synthesis and secretion of CRH and ACTH, respectively) to form a feedback regulation loop. AEF has a certain regulatory effect on the hormone level in pigeons, which is likely to affect part of the pathway in the HPA axis. Under stress conditions, the connection mechanism between the changes of AEF on blood chemistry and hormone levels and pigeon performance remains to be established, and the impact of stress on HPA and any possible adaptation of AEF during stress remain to be studied.

Previous studies showed that Ligustrum lucidum effected on laying performance and egg quality (Li et al., 2017). However, the mechanism of comprehensive influence on egg laying performance is not completely clear. Chinese herbal extracts improved the feed conversion rate (such as astragalus extract), by promoting the proliferation of beneficial bacteria and helping the intestinal tract absorb nutrients (Liu et al., 2021). Adding tea polyphenols to the diet also improved the protein quality of hens (Zhou et al., 2021). Egg quality is mainly evaluated by egg weight, Haugh unit, eggshell strength, eggshell thickness, eggshell color, and yolk color. Eggshell thickness affects egg breakage rate. Calcium is the main component of eggshell. The quality of eggshell formation directly depends on absorption and the utilization of calcium by layers (Huang et al., 2022). Recent studies have shown that trace elements (manganese, zinc) and feed additives and plant extracts can regulate eggshell quality (Lokaewmanee et al., 2013; Boka et al., 2014; Zhang et al., 2017). Our experiments show that the thickness of pigeon eggshell increases significantly, which may affect the absorption and utilization of mineral elements and regulate the mineralization of eggshell. Egg shell quality

and improvement measures are based on the health of digestive tract. H&E staining showed that stress break intestinal villi and affect the development of villi, while AEF repaired the shortening and breaking of intestinal villi caused by stress to a certain extent. The digestion and absorption of calcium and phosphorus in gastrointestinal health will be balanced, and the calcium supplied to the fallopian tubes will increase to improve the quality of eggs.

The small intestine is the most important place for animals to digest and absorb nutrients. Intestinal morphological characteristics such as villi height, recess depth, and V/C provided information on the function and health of the intestinal mucosa. Pigeon stress has an impact on intestinal villi thickness and surface area which resulted in a reduction in the rate at which intestinal epithelial cells mature (Boylu et al., 2005). The increased intestinal mucosal absorption function and improved intestinal mucosal structure are both reflected in the high V/C ratio (Xu et al., 2022b). In this study, the supplement of drinking AEF increased the recess depth, villi height of duodenum, jejunum, and ileum and V/C of jejunum. Results indicated that AEF repaired the villi height of intestinal mucosa in stressed pigeons. We speculate that AEF could improve the proliferation and differentiation of intestinal epithelial cells and the renewal rate of intestinal stem cells. At the same time, AEF promoted the expression of Claudin-3 and Claudin-4 in the small intestine of stressed pigeons. They indicated that AEF enhanced the integrity of mucous layer, produced a host friendly intestinal environment and resisted pathogen infection.

Microorganisms alter the host's immune system, digestion, and adaptability, all of which have a variety of negative effects on the host's health and ability to survive (Zhang et al., 2019). Oxidative stress results from a significant rise in ROS levels in vivo caused by an increase in metabolic activity. Excessive ROS will lead to intestinal structural damage, ischemia and hypoxia damage. Disorders in nutrient absorption come from the ease with which the intestinal flora's balance was damaged (Assimakopoulos et al., 2004). In addition, oxidative stress acts on the intestinal flora by changing the intestinal environment, resulting in the proliferation of a large number of opportunistic pathogens (such as *Escherichia coli, Streptococcus faecalis*, and *Staphylococcus*) and inhibiting probiotics (such as *Lactobacillus* and *Bifidobacterium*) (Wang et al., 2022). Beneficial bacteria maintained the homeostasis of intestinal oxidation and antioxidant system and corresponding metabolites. Our research showed that *Firmicum* was the main abundant bacteria in the pigeon colon microbiota, accounting for more than 60% of the total bacteria. *Firmicum* is mainly involved in the absorption of dietary energy by the host and the conversion of polysaccharides into absorbable monosaccharides and short chain fatty acids. The relative abundance of *Firmicum* and *Proteus* changed at the gate level as a result of AEF addition in drinking water, which is beneficial for intestinal health. At the family level, AEF supplementation increased the abundance of lactic acid bacteria and reduce the abundance of *Trichomonas. Lactobacilli* is an important probiotic that promote intestinal health, enhance growth, development of immune cells, tissues, and organs and immune capacity (Yang et al., 2019). It was discovered that *Lactobacillus* metabolites lowered gut pH, which prevented the growth of harmful bacteria (Heeney et al., 2019). Instead, we studied showed that AEF had no effect on the number of OTUs.

Hematological and biochemical blood indices under stressful circumstances represent the physiological state of pigeons, and the content of immunoglobulins in serum can reflect the protein metabolism of the body. TP and ALB can maintain blood osmotic pressure, GLB participates in coagulation regulation and humoral immunity, and has anti-inflammatory and bacteriostatic effects, repair damage, and improve body immunity. Adding plant extracts to the diet can enhance the body immunity and resist high temperature stress by increasing the serum GLB content of roosters (Attia et al., 2019). Clinically, ALT and AST are important indicators reflecting liver function (Ahmed et al., 2018). When liver dysfunction or hepatocytes are damaged, ALT and AST activities will increase significantly (Xu et al., 2021). In stressed pigeons, Astragalus membranaceus and Ligustrum lucidum administration decreased blood levels of ALT, demonstrating their significance in reducing the detrimental effects of heat stress (Lin et al., 2007; Abdelli et al., 2021; Zhou et al., 2021a). The results of this test are similar to those of previous studies. After AEF is given, the content of GLB in serum increases, and the low A/G indicates that the immunity decreases, while AEF increases the A/G. The contents of ALT and AST increased in the stress group, and ALT in serum decreased significantly after administration.

Practice has proved that under stress, the productivity (egg laying rate, egg quality, fertilization rate, weight gain, etc.) dan health status of pigeons will be significantly reduced(Chen et al., 2021). And the immune biological index of pigeons will be significantly reduced, and many diseases will occur (Xu et al., 2022a). Astragalus polysaccharide can improve the farrowing performance of sows, improve the survival rate of piglets, improve the milk production and milk quality (Wu et al., 2021). After adding Astragalus membranaceus to broiler feed, the growth rate and feed conversion rate of broiler were promoted (Qiao et al., 2022). Adding 0.25% Ligustrum lucidum to the laying hens' diet can increase the average egg weight and protein height and reduce the oxidative stress of the laying hens' fallopian tubes (Li et al., 2017). Adding Epimedium flavonoids can significantly increase the selected reproductive features in layer hens (Huo et al., 2020). However, Chinese medicine combination remain poorly understood. Results showed that inclusion of natural feed additives (plant extracts) in the diet of stressed pigeons mitigated the adverse impact of stress on the growth performance. Furthermore, AEF significantly improved body weight gain, average daily gain, average daily feed intake. The pH, yellowness of meat, dripping loss and cooking loss were changed in 14-

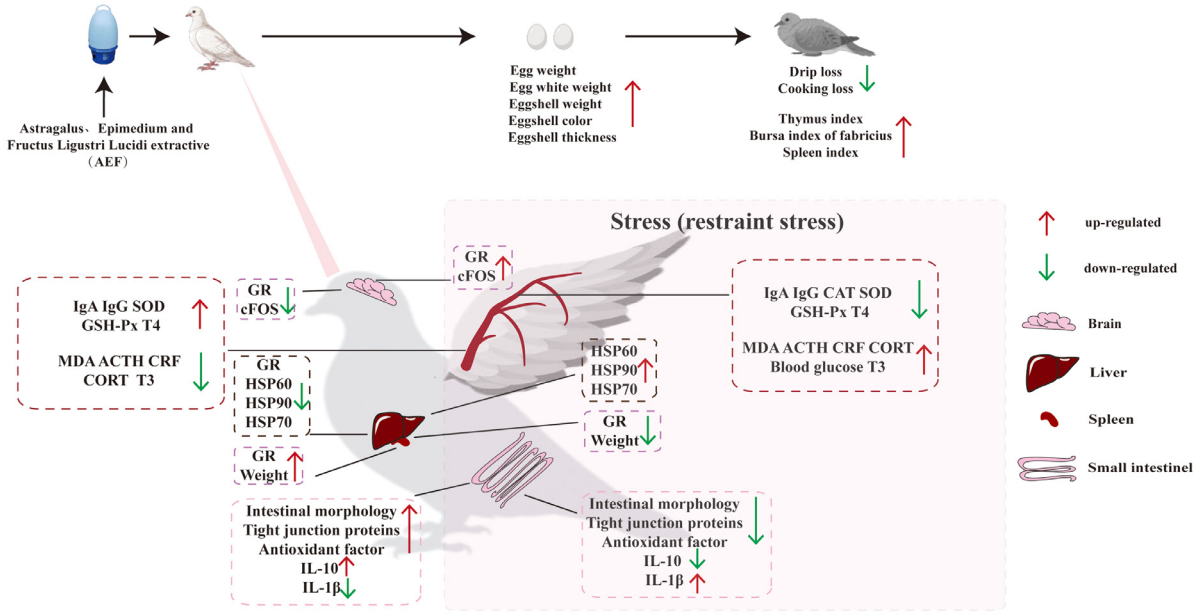

Figure 6. The overall picture shows the main results obtained in the current work. The up arrows (↑) indicate increasing effects, and the down arrow (↓) indicates decreasing effects.

day-old or 21-day-old squab under stress. AEF supplementation reduced dripping loss and cooking loss.

CONCLUSIONS

This study showed that adding AEF extract to drinking water enhanced the intestinal health and growth performance of stressed pigeons. Treatment with AEF enhanced intestinal bacterial composition, intestinal morphology, immune responses and growth performance in pigeons under stress. Furthermore, AEF exhibited greater impact on immune response, gut microbiota, antioxidant capacity and overall performance of stressed pigeons. AEF can be used as a health product to improve pigeon stress. This study provided an important contribution to the sustainable development of the livestock and poultry industry. With the large-scale and intensive development of pigeon breeding and the improvement of production level, stress factors have an important impact on the health and productivity of pigeons (Figure 6).

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DISCLOSURES

The authors declare no conflict of interest.

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