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Effects of phytogenic feed additives on the growth, blood biochemistry, and caecal microorganisms of White Roman geese

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Abstract: This study investigated the effects of *Lycium chinense* Miller stem (LCM) and *Origanum vulgare* Linn (oregano) essential oil on growth, blood biochemistry, and caecal microorganisms in White Roman goose. A total of 96-day-old White Roman geese were randomly allocated to a control group and three treatment groups. The G 0.05 diet was enriched with 0.05% LCM extract, the G 0.5 diet was enriched with 0.5% LCM extract, and the O 0.05 diet was enriched with 0.05% oregano essential oil. Each treatment was allotted to three pens, with four males and four females per pen. Geese were fed for 83 days. Geese in group O 0.05 had greater body weight gain than those in groups G 0.05 and G 0.5 in the first 28 days ($P < 0.05$), whereas the geese in group O 0.05 also showed greater body weight gain than those in control and G 0.05 groups in total 83 days ($P < 0.05$). No significant differences in serum creatinine, glutamic oxaloacetic transaminase, glutamic-pyruvic transaminase, cholesterol, low-density lipoprotein, or high-density lipoprotein were observed. Serum glutathione peroxidase concentrations were significantly higher for geese in group G 0.5 ($P < 0.05$) than for the control group at 83 days of age. At 87days of age, one male and one female per pen were slaughtered, and the caecum contents were collected for gene sequencing by 16S ribosomes. No significant differences in microbial alpha diversity were observed. In conclusion, supplementation with 0.05% oregano essential oil increased body weight gain for 83 days. In addition, supplementation with 0.5% LCM extract increased glutathione peroxidase activity at 83 days of age ($P < 0.05$). In summary, our study showed that phytogenic feed additives improve growth and have antioxidant and immune properties in geese.

Keywords: *Lycium chinense* Miller; glutathione peroxidase; antioxidant; immune property

Phytogenics are the chemical constituents naturally present in various plants, such as herbs, spices, and essential oils and their derivatives (Windisch et al. 2008). Because of their high pharmacological activity, phytobiotics are a promising natural alternative to antibiotic growth promoters, which

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the European Union has banned. Moreover, phytogenic feed additives are a more natural, safer, and healthier supplement for promoting growth than antibiotics (Grashorn 2010; Sadek et al. 2014). Plant extracts can promote the growth of probiotics in the host gut, increase concentrations of short-chain fatty acids in the host ileum and colon, and reduce the production of potentially harmful microorganisms and protein metabolites. Xu et al. (2017) found differences in the caecum and faecal bacteria of Yangzhou geese on a whole-grass diet versus those fed a high-grain diet. Diet composition changes the intestinal bacteria of geese, which in turn affects their digestion and health.

Lycium is a perennial shrub or tree of the genus *Lycium* in the family Solanaceae and is categorised as fruit or leaf. Fruit species include *L. barbarum* L. and *L. dasystemum* P., and leaf species include *L. chinense* Miller (LCM). The stems and leaves of LCM are rich in polyphenols, such as flavonoids and glycoconjugates, and have antibacterial, antioxidant, superoxide anion, and anti-inflammatory properties (including the inhibition of nitric oxide and inflammatory factors IL-6 and TNF-α; Chung et al. 2013; Mocan et al. 2015) and an anti-inflammatory effect on human kidney cells. Olatunji et al. (2015) found that the aerial part of LCM significantly ($P < 0.05$) reduced the concentration of inflammatory factors such as TNF-α, IL-6, and IL-1 in the serum of mice and was effective in attenuating gastric ulcers. The results of antimicrobial tests have demonstrated that LCM extracts have antibacterial effects against gram-positive and gram-negative bacteria and fungi (Lee et al. 2004; Mocan et al. 2014); however, few studies have investigated the effect of LCM extracts in poultry.

Origanum vulgare Linn. is a perennial herb of the genus *Origanum* in the Lamiaceae family and is also known as Dianthus, Oregon leaf, or wild horse tulip. The main chemical constituents of oregano are cymophenol and thymol, which often have strong H-donating activity thus making them extremely effective antioxidants (Brewer 2011). Another antimicrobial test demonstrated that *O. vulgare* Linn (oregano) essential oil has significant antimicrobial activity against bacteria, fungi, and yeasts (Sahin et al. 2004). The combined supplementation of oregano and garlic essential oils had a potent anticoccidial effect *in vitro* and a growth-promoting effect in broilers (Sidiropoulou et al. 2020). In a study on broilers, the use of oregano powder and oregano oil as feed additives significantly ($P < 0.05$) increased daily weight gain and reduced the number of Enterobacteriaceae and *Escherichia coli* in the intestinal tract (Vlaicu et al. 2020).

The application of phytogenic feed additives to geese diet is relatively uncommon. The application of phytogenic feed additives to geese diet is relatively uncommon. The previous study showed growing ducks fed 3%, 6%, 9% alfalfa meal diets were not significantly different in average daily gain from those fed no alfalfa diet ($P > 0.05$) (Jiang et al. 2012). Similar results were also observed in geese study. Cheeke and his colleagues (Cheeke et al. 1983) demonstrated that geese discriminated against the alfalfa-containing diet at 2.5% alfalfa and not showed discrimination at alfalfa levels of 0.5% and 1% ($P < 0.05$). Hence, in our study, the content of test feed was included 99% basic diet and 1% phytogenic feed additives with alfalfa meal. The total of phytogenic feed additives and alfalfa meal should be 1%. This study investigated the effects of different phytogenic feed additives on the growth, blood biochemistry, and intestinal microorganisms of White Roman geese over 83 days. The results can be used as a reference for the application of phytogenic feed additives in the domestic goose breeding industry.

MATERIAL AMD METHODS

Animal care

This study was conducted from March to June 2020 at the Changhua Animal Propagation Station of the Livestock Research Institute, Council of Agriculture, Taiwan (23°51'19" N 120°33'27"E), located 39 m above sea level. The study was approved by the Institutional Animal Care and Use Committee at the Changhua Animal Propagation Station of the Livestock Research Institute, Council of Agriculture (Case No. LRICH IACUC 10907).

Study participants and diet

In total, 96 White Roman geese bred at the Changhua Animal Propagation Station of the Livestock Research Institute were included. Geese were raised in an indoor house with plastic perforated floors and natural lighting. Food and water were given *ad libitum* during the study. The study was divided into three stages ac-

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cording to the age of the geese: starting (1 to 28 days), growing (29 to 57 days), and fattening (58 to 83 days). The diets used in the study were designed according to the standards of NRC (1994). The composition of the diets and the general composition of the phytogenic feed additives for White Roman geese are shown in Tables 1 and 2.

Data collection and analysis

In total, 96 White Roman day-old geese were randomly allocated to four groups, namely a control group (without additional supplementation) and three experimental treatment groups (G 0.05, G 0.5, and O 0.05), the basal diet of which was enriched by supplementation as follows: the G 0.05 diet was enriched with 0.05% LCM extract and 0.95% alfalfa meal, the G 0.5 diet was enriched with 0.5% LCM extract and 0.5% alfalfa meal, and the O 0.05 diet was enriched with 0.05% oregano essential oil and 0.95% alfalfa meal. Geese were divided into pens, each containing four males and four females. LCM was planted, harvested, and dried at the Miaoli District Agricultural Research and Extension Station, Council of Agriculture, Executive Yuan. Extract

Table 1. The composition of phytogenic additives experimental diets for White Roman geese

Ingredients	Starting (1–28 days)				Growing (29–57 days)				Fattening (58–83 days)			
	C	O 0.05	G 0.05	G 0.5	C	O 0.05	G 0.05	G 0.5	C	O 0.05	G 0.05	G 0.5
Yellow corn	30.46	30.46	30.46	30.46	31.78	31.78	31.78	31.78	69.30	69.30	69.30	69.30
Brown rice (rough)	30.46	30.46	30.46	30.46	31.78	31.78	31.78	31.78	–	–	–	–
Soybean meal	28.71	28.71	28.71	28.71	21.29	21.29	21.29	21.29	20.79	20.79	20.79	20.79
ELCM powder	–	–	0.05	0.50	–	–	0.05	0.50	–	–	0.05	0.50
EOE powder	–	0.05	–	–	–	0.05	–	–	–	0.05	–	–
Alfalfa meal	1.00	0.95	0.95	0.50	1.00	0.95	0.95	0.50	1.00	0.95	0.95	0.50
Rice hull	–	–	–	–	2.97	2.97	2.97	2.97	3.86	3.86	3.86	3.86
Wheat bran	–	–	–	–	4.94	4.94	4.94	4.94	–	–	–	–
Fish meal	3.47	3.47	3.47	3.47	–	–	–	–	–	–	–	–
Molasses	2.97	2.97	2.97	2.97	2.97	2.97	2.97	2.97	1.98	1.98	1.98	1.98
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.29	0.29	0.29	0.29
Dicalcium phosphate	1.29	1.29	1.29	1.29	1.58	1.58	1.58	1.58	1.19	1.19	1.19	1.19
Limestone (pulverised)	0.69	0.69	0.69	0.69	0.79	0.79	0.79	0.79	0.69	0.69	0.69	0.69
Choline chloride (50%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.25	0.25	0.25	0.25	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine-HCl	–	–	–	–	–	–	–	–	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.20	0.20	0.20	0.20
Mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values												
Crude protein (%)	19.69	19.69	19.69	19.63	15.66	15.66	15.66	15.60	15.54	15.53	15.53	15.48
ME (kcal/kg)	2 734	2 734	2 734	2 728	2 646	2 645	2 645	2 640	2 789	2 789	2 789	2 783
Calcium (%)	0.88	0.88	0.88	0.87	0.81	0.81	0.81	0.80	0.66	0.66	0.66	0.65
Non-phytate phosphorus (%)	0.47	0.47	0.47	0.46	0.43	0.43	0.43	0.43	0.35	0.35	0.35	0.34
Crude fibre (%)	2.90	2.89	2.89	2.77	4.36	4.35	4.35	4.23	4.55	4.54	4.53	4.41

C = blank trial; ELCM powder = dry powder of *Lycium chinense* Miller extract; EOE powder = dry powder of volatile oil of *Origanum valgare* Linn. extract; G 0.05 = ELCM 0.05%; G 0.5 = ELCM 0.5%; ME = metabolisable energy; O 0.05 = EOE 0.05%

[1]Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 20 IU; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3mg; vitamin B_{12}, 30 µg; biotin, 200 µg; vitamin K_3, 3 mg; niacin, 30 mg; folic acid, 2 mg and pantothenic acid, 10 mg

[2]Supplied per kilogram of diet: Cu, 30 mg; Fe, 200 mg; Zn, 100 mg; Mn, 160 mg; Co, 500 µg; I, 1.7 mg and Se, 300 µg

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Table 2. Proximate components analysis of phytogenic additives (dry matter)

Item	ELCM powder	EOE powder
Moisture (%)	5.15	15.57
Crude ash (%)	5.01	7.53
Crude protein (%)	4.35	14.21
Crude fat (%)	0.00	3.82
Crude fiber (%)	0.39	5.61
Nitrogen – free extract (%)	85.01	53.26
Selenium (ppm)	0.80	0.77

ELCM powder = dry powder of *Lycium chinense* Miller extract; EOE powder = dry powder of volatile oil of *Origanum valgare* Linn. extract

of LCM was prepared by I Chuan Biotechnology Co., Ltd. (Tainan, Taiwan). The LCM was cultivated on the flatland of Miaoli County, Taiwan. The analysis of soil quality included nutrient cations (Ca, K, Mg), P and electronic conductivity were 1 294.33 ppm, 186.00 ppm, 93.67 ppm, 42.67 ppm and 0.11 ds/m, respectively. The plant materials were harvested in September 2019, coarsely crushed and dried at 50 °C. The dried materials were boiled in 100 °C water for 3 h with a ratio of 1 : 10, then spray-dried into powder. Oregano essential oil feed additive (ORSENTIAL™ Dry) was purchased from Kemin Industries Asia Pte Ltd (Des Moines, IA, USA). The geese were fed food and water *ad libitum* for 83 days, feed intake was collected weekly, and body weights were recorded at 28, 57, and 83 days of age to calculate the feed conversion ratio.

Blood samples were centrifuged at 4 °C for 15 min at 1 610 × *g* (Universal 320R, Hettich, Germany). Serum was collected and analysed using a Lanner T-900 blood biochemistry analyser (Lanner Biotechnology Co., Ltd., Taichung, Taiwan) with reagents of the same brand. Creatinine (CREA), glutamic oxaloacetic transaminase, glutamic-pyruvic transaminase, cholesterol (CHOL), triglyceride (TG), high-density lipoprotein, and low-density lipoprotein concentrations were analysed. Immunoglobulin G (IgG) concentrations were analysed using a Nori goose IgG ELISA kit (Genorise, Glen Mills, PA, USA). Glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD) activities were analysed using an automatic biochemistry analyser (Hitachi 7150, Tokyo, Japan).

At 87 days of age, one male and one female per pen were slaughtered, and the contents of the caeca of the slaughtered geese were collected. The contents were placed into 15-mL sterilised conical plastic centrifuge tubes (Greiner Bio-One, Kremsmünster, Austria) with dry ice. Microbial genomic DNA were extracted from the caecum contents using a QIAmp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) by gene sequencing with 16S ribosomes. Sequencing, sequence splicing, filtering, and clustering of operational taxonomic units was commissioned by the Microbial Research Center (Biotools Co., Ltd, New Taipei City, Taiwan). For sequence analysis, the 16S ribosomal amplicon sequencing generated 300 bp paired-end raw reads assembled using FLASH v.1.2.11 (Magoc and Salzberg 2011). De-multiplexing was performed based on barcode identification (Caporaso et al. 2010), and reads with a Q score less than 20 were discarded (Caporaso et al. 2010). Reads were filtered for chimeras using UCHIME (Edgar et al. 2011; Haas et al. 2011), then clustered into OTUs at 97% sequence identity using UPARSE in USEARCH v.7 pipeline. Taxonomy classification was annotated using the RDP classifier v.2.2 (https://bioweb.pasteur.fr/packages/pack@rdp_classifier@2.2) algorithm with information from the Silva Database v.132 (https://www.arb-silva.de/documentation/release-132/). The samples were grouped according to treatment and analysed at different taxonomic levels. Kruskal-Wallis test was used to identify significant differences in relative abundance between the control and sample of interest.

Statistical analysis

Data were analysed using ANOVA in SAS v9.3 (SAS Institute, Inc., NC, USA). The significance of differences among the treatments was evaluated using the least significance difference test, and statistical significance was indicated if $P < 0.05$.

RESULTS AND DISCUSSION

Effects of phytogenic feed additives on the growth performances

The effects of phytogenic feed additives on growth in White Roman geese at one to 83 days of age is showed in Table 3. Body weight gain during the starting stage was higher among geese in the

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Table 3. Effects of phytogenic additives on growth performances in White Roman geese from 1 to 83 days of age

Days of age	Phytogenic additives supplement			
	C	O 0.05	G 0.05	G 0.5
Body weight (kg/bird)				
1	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
28	2.32 ± 0.04	2.34 ± 0.13	2.22 ± 0.04	2.26 ± 0.01
57	4.29 ± 0.04	4.45 ± 0.06	4.19 ± 0.05	4.28 ± 0.08
83	5.00 ± 0.11	5.21 ± 0.16	4.86 ± 0.10	4.91 ± 0.13
Body weight gain (kg/bird)				
1–28	2.20 ± 0.03[ab]	2.22 ± 0.03[a]	2.11 ± 0.03[c]	2.15 ± 0.01[b]
29–57	1.98 ± 0.13	2.12 ± 0.08	1.97 ± 0.03	2.02 ± 0.08
58–83	0.70 ± 0.06	0.76 ± 0.13	0.70 ± 0.12	0.64 ± 0.06
1–83	4.78 ± 0.26[b]	5.10 ± 0.16[a]	4.74 ± 0.10[b]	4.80 ± 0.13[ab]
Feed intake (kg/bird)				
1–28	3.88 ± 0.02	3.81 ± 0.10	3.78 ± 0.14	3.94 ± 0.08
29–57	8.33 ± 0.23	8.50 ± 0.52	8.00 ± 0.49	8.18 ± 0.39
58–83	6.70 ± 0.21[b]	7.21 ± 0.42[a]	6.73 ± 0.17[ab]	6.67 ± 0.14[b]
1–83	18.91 ± 0.41	19.52 ± 0.97	18.51 ± 0.79	18.79 ± 0.44
Feed conversion ratio (kg feed/kg gain)				
1–28	1.76 ± 0.03[ab]	1.71 ± 0.02[b]	1.80 ± 0.05[a]	1.83 ± 0.04[a]
29–57	4.22 ± 0.30	4.02 ± 0.21	4.06 ± 0.28	4.07 ± 0.22
58–83	9.61 ± 0.96	9.65 ± 1.39	9.75 ± 1.48	10.58 ± 0.82
1–83	3.97 ± 0.28	3.83 ± 0.07	3.90 ± 0.12	3.92 ± 0.09

C = blank trial; G 0.05 = *Lycium chinense* Miller extract 0.05%; G 0.5 = *Lycium chinense* Miller extract 0.5%; O 0.05 = *Origanum valgare* Linn. extract 0.05%

[a–c]Means in the same row without a common superscript differ significantly ($P < 0.05$)

Means ± standard deviation ($n = 3$)

O 0.05 group than among geese in the G 0.05 and G 0.5 groups, and body weight gain from the start of stage one to the end of stage three was higher among geese in the O 0.05 group than among geese in the G 0.05 and control groups ($P < 0.05$). During the fattening stage, the feed intake of the O 0.05 group was significantly ($P < 0.05$) higher than that of the G 0.5 and control groups. The positive effects of oregano essential oil have been proven on growth performances in terms of body weight gain and feeding efficiency (Ertas et al. 2005; Brenes and Roura 2010). A recent study also observed similar results of *O. vulgare* in white broiler chickens, which were fed basal diet enriched with 0.01% *O. vulgare* oil or a basal diet enriched with 0.005% *O. vulgare* oil and 1% *O. vulgare* powder had significantly higher overall body weight gain than those fed a nonenriched basal diet at 14 to 42 days of age ($P < 0.05$).

In contrast, the daily feed intake was not significantly different between the treatments and control groups (Vlaicu et al. 2020). In addition, the feed conversion ratio of the O 0.05 group was significantly lower than that of the G 0.05 and G 0.5 groups during the starting stage ($P < 0.05$). Ertas et al. (2005) demonstrated that the essential oils of oregano, clove, and anise could be considered potential natural growth promoters in poultry. In this study, the feed conversion ratio was improved in broiler chickens fed a diet of 200 parts per million essential oils of oregano, clove, and anise by approximately 12% more than the control group and approximately 6% more than the group fed antibiotics. Another study also observed a positive result when 100 mg/kg of essential oils of rosemary and oregano supplementary to the diet increased the body weight of white broilers (Mathlouthi et al. 2012). In general, oregano seems to have a positive effect on the growth of poultry. Our findings are consistent with those of previous studies. We found that oregano essential oil increased the body weight of geese at one to 83 days of age, increased feed intake during the fattening stage, and improved the feed conversion ratio during the starting stage. However, the LCM extract had no significant effect on the growth of geese (Table 3). These results were also revealed in Shen's study (Shen et al. 2020), which revealed no adverse effects on growth and blood biochemistry when feeding a concentrate supplemented with 14.9% fresh LCM (on a fresh weight basis) and 5% LCM lignified stalk powder at 83 days of age.

Effects of phytogenic feed additives on blood biochemistry

The effects of phytogenic feed additives on blood biochemistry in White Roman geese at 83 days of age are shown in Table 4. Serum IgG concentrations were significantly higher among the geese in the G 0.05 and G 0.5 groups than in the O 0.05 group ($P < 0.05$). Moreover, serum GPx activity was significantly higher among geese in the G 0.5 group than in the control group ($P < 0.05$). Phytogenic feed additives such as LCM have radical scavenging properties against the stable synthetic DPPH (1,1-diphenyl-2-picrylhydrazyl) radical (Chung et al. 2013; Mocan et al. 2015). Wang et al.

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Table 4. Effects of phytogenic additives on blood biochemical parameters in White Roman geese at 83 days of age

Parameter	Phytogenic additives supplement			
	C	O 0.05	G 0.05	G 0.5
TG (mg/dl)	95.67 ± 16.84	100.50 ± 18.60	99.33 ± 15.64	107.83 ± 15.30
CHOL (mg/dl)	172.83 ± 15.18	159.17 ± 11.32	167.83 ± 20.84	156.33 ± 25.67
GOT (IU/l)	13.83 ± 4.26	9.83 ± 2.64	13.50 ± 5.17	14.33 ± 6.31
GPT (IU/l)	9.50 ± 1.05	11.00 ± 0.89	10.33 ± 2.34	11.33 ± 1.86
CREA (mg/dl)	0.28 ± 0.08	0.27 ± 0.07	0.25 ± 0.06	0.23 ± 0.08
HDL (mg/dl)	81.83 ± 7.33	77.50 ± 6.80	78.83 ± 6.85	74.00 ± 10.47
LDL (mg/dl)	75.17 ± 5.71	67.50 ± 5.50	76.17 ± 10.91	67.67 ± 14.76
IgG (mg/ml)	0.48 ± 0.03^{a}	0.38 ± 0.06^{b}	0.48 ± 0.04^{a}	0.44 ± 0.06^{a}
CAT (nmol/min/ml)	5.96 ± 0.68	4.45 ± 1.51	4.82 ± 2.39	5.08 ± 2.26
SOD (IU/ml)	1.61 ± 0.15	1.70 ± 0.40	1.62 ± 0.83	1.38 ± 0.30
GPx (IU/g Hb)	$1\,089.33 \pm 227.31^{b}$	$1\,150.50 \pm 234.28^{ab}$	$1\,150.33 \pm 147.76^{ab}$	$1\,424.83 \pm 372.93^{a}$

C = blank trial; CAT = catalase; CHOL = cholesterol; CREA = creatinine; G 0.05 = *Lycium chinense* Miller extract 0.05%; G 0.5 = *Lycium chinense* Miller extract 0.5%; GOT = glutamic oxaloacetic transaminase; GPT = glutamic -pyruvic transaminase; GPx = glutathione peroxidase; HDL = high density lipoprotein; IgG = immunoglobulin G; LDL = low density lipoprotein; O 0.05 = *Origanum valgare* Linn. extract 0.05%; SOD = superoxide dismutase; TG = triglyceride

^{a,b}Means in the same row without a common superscript differ significantly ($P < 0.05$)

Means ± standard deviation ($n = 6$)

(2011) and Li et al. (2017) demonstrated that adding selenium to the basal diet of geese and ducks has no significant effects on their slaughter performance and growth performance but improves serum immune biochemical indicators and antioxidant capacity. The selenium content of LCM is approximately 0.38–1.72 ppm. The selenium content of the LCM extract used in this study was 0.80 ppm. The selenium in the LCM extract used in this study had no significant effect on the growth performance of geese. In contrast, it increased the activity of GPx, similar to a previous study (Olatunji et al. 2015). Pretreatment with the aerial parts of *L. chinense* exerts gastroprotective properties against ethanol-induced gastric ulcer in mice models. The underlying mechanisms involved in the antiulcerogenic effects might be related to the scavenging of oxidative free radicals, the upregulation of antioxidants, the anti-inflammatory status, the reduced expression of proinflammatory mediators, and malondialdehyde lipid peroxide levels. In addition, previous studies also found that supplementation of 0.5% and 1% oregano powder in the diet can significantly increase the SOD activity in the serum of meat ducks, and the GPx activity in the breast meat tissue was also significantly increased compared with the antibiotic-treated group (Park et al. 2015). Yi (2000) showed that adding LCM powder to the diet of rats, the concentration of TG and CHOL in the serum decreased significantly, indicating that LCM has a hypolipidemic effect. Our study found no significant effect on serum CHOL and TG concentrations, possibly due to the amount of feed additive used and the different species. Thyme essential oil promotes protein metabolism in broilers, enhances lipolysis, and improves immune status. It can be considered a potential growth enhancer for broilers. On the other hand, it satisfies consumers who desire environmentally friendly farming conditions (Zhu et al. 2014).

Effects of phytogenic feed additives on the caecal microorganisms

The effects of phytogenic feed additives on the alpha diversity of caecal microbes in White Roman geese at 87 days of age are shown in Table 5. No significant difference between the treatment groups and the control group in terms of Shannon index, Simpson index, abundance-based coverage estimator, or Chao1 estimator were observed.

Figure 1 shows the effects of phytogenic feed additives on bacterial flora at the phylum level of the caecal contents in White Roman geese at 87 days of age. Bacteroidetes and Firmicutes

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Table 5. Effects of phytogenic additives on comparison of alpha diversity of caecal microbes in White Roman geese at 87 days of age

Parameter	Phytogenic additives supplement			
	C	O 0.05	G 0.05	G 0.5
Shannon index	6.48 ± 0.21	6.50 ± 0.10	6.57 ± 0.31	6.77 ± 0.23
Simpson index	0.956 ± 0.009	0.974 ± 0.002	0.974 ± 0.005	0.980 ± 0.003
ACE	618.55 ± 25.70	622.22 ± 53.45	644.50 ± 32.36	639.78 ± 24.27
Chao1	631.12 ± 33.64	631.23 ± 59.29	644.39 ± 31.17	645.65 ± 24.76

ACE = abundance-based coverage estimator; C = blank trial; G 0.05 = *Lycium chinense* Miller extract 0.05%; G 0.5 = *Lycium chinense* Miller extract 0.5%; O 0.05 = *Origanum valgare* Linn. extract 0.05%

Means ± standard deviation (*n* = 6); there is no significant difference in the traits between treatments

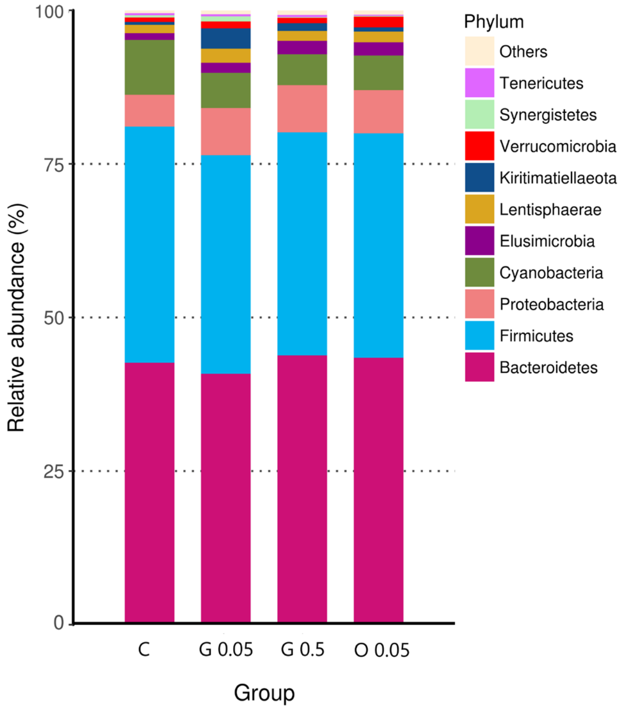

Figure 1. Effects of feeding phytogenic additives on bacterial flora at the phylum level of the caecal contents in White Roman geese at 87 days of age

C = blank trial; G 0.05 = *Lycium chinense* Miller extract 0.05%; G 0.5 = *Lycium chinense* Miller extract 0.5%; O 0.05 = *Origanum valgare* Linn. extract 0.05%

dominated the bacterial flora analysis, with a total relative abundance of more than 75%; in terms of abundance, these phyla were followed by Proteobacteria, Cyanobacteria, Elusimicrobia, Lentisphaerae, Kiritimatiellaeota, Verrucomicrobia, Synergistetes, and Tenericutes. Vlaicu et al. (2020) found that daily weight gain was significantly higher among test subjects in the treatment groups E1 (0.01% oregano oil) and E2 (0.005% oregano oil and 1% oregano powder) than among subjects in the control group and that daily feed intake was comparable between the three groups ($P < 0.05$). Enterobacteriaceae and *E. coli* were significantly less abundant in the intestines of the subjects of the treatment groups than in those of the control group. In contrast, *Lactobacillus* spp. were significantly more abundant in the intestines of the subjects in the treatment groups than in the control group ($P < 0.05$). Our result is similar to Liu (2017), which showed that oregano essential oil significantly increased the abundance of *Lactobacillus* colonies in the caeca of broiler chickens ($P < 0.05$). Per analysis of genus-level microbes in the geese at 87 days of age, the geese in the O 0.05 group had a higher abundance of *Elusimicrobium* and *Phascolarctobacterium* than the geese in the control group (Figure 2A). Cheng et al. (2022) observed the same case, which found that the caeca of test subjects that were fed either 0.05 or 0.75 g/kg of an essential oil-palygorskite composite had a significantly higher abundance of *Elumicrobium* than the caeca of subjects in the control group at the genus level ($P < 0.05$). *Phascolarctobacteria* produce short-chain fatty acids, including acetate and propionate, and are associated with the metabolic state and mood of the host (Wu et al. 2017). Figure 2B shows that *Blautia* was more abundant in the geese in group G 0.05 than in the geese in the control group. *Blautia* is a genus of anaerobic bacteria with probiotic characteristics that occurs in the faeces and intestines of mammals. As a dominant genus of intestinal microbiota, *Blautia* plays specific roles in metabolic diseases, inflammatory diseases, and biotransformation (Liu et al. 2021). At the genus level, *Blautia* was the only gut mi-

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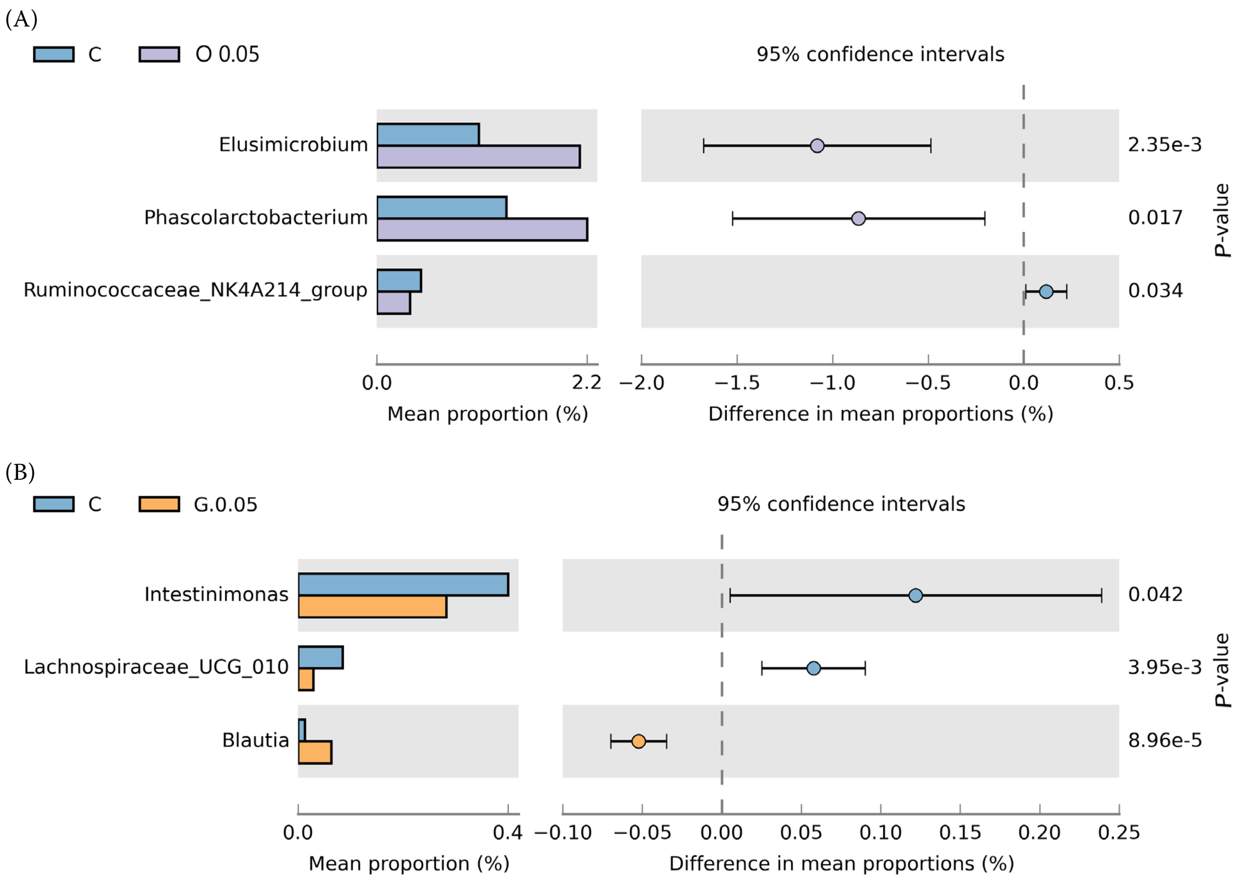

Figure 2. Effects of feeding phytogenic additives on comparison test at the genus level of caecal microbes in White Roman geese at 87 days of age

(A) Comparison of O 0.05 and C; (B) comparison of G 0.05 and C

C = blank trial; G 0.05 = *Lycium chinense* Miller extract 0.05%; O 0.05 = *Origanum valgare* Linn. extract 0.05%

crobe significantly and inversely associated with visceral fat (Ozato et al. 2019). *Intestinimonas* and *Lachnospiraceae* were more abundant in geese in the control group than in geese in group G 0.05. Some genera of *Intestinimonas* have been found to produce short-chain fatty acids (Zhou et al. 2019), which are important energy sources and signaling molecules for intestinal bacteria to maintain the intestinal barrier and regulate the immune system (Jin et al. 2019).

CONCLUSION

In this study, we enriched the diets of White Roman geese with phytogenic feed additives. LCM extract did not significantly affect growth but increased serum IgG concentrations and GPx activity, eliciting positive antioxidant effects and immune function. Supplementation with 0.05% oregano essential oil significantly increased body weight, increased feed intake during the fattening stage, and improved the feed conversion ratio during the starting stage. Oregano essential oil had a positive effect on growth. In summary, in the present study, the LCM extract and oregano essential oil had no significant effect on the alpha diversity of caecal microbes in the geese at 87 days of age but affected caecal microbes at the genus level. Additional studies on the effects of phytogenic feed additives on the caecal microbes and the health of geese are warranted.

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Conflict of interest

The authors declare no conflict of interest.

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