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Comparative effects of dietary herbal mixture or guanidinoacetic acid supplementation on growth performance, cecal microbiota, blood profile, excreta gas emission, and meat quality in Hanhyup-3-ho chicken

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ABSTRACT Phytogenic feed additives are renowned for their growth promotion, gut health enhancement, and disease prevention properties, which is important factors for sustaining prolonged poultry rearing. The study aimed to evaluate the effect of herbal mixture (mixture of ginseng and artichoke) or guanidinoacetic acid (**GAA**) on growth performance, cecal microbiota, excretal gas emission, blood profile, and meat quality in Hanhyup-3-ho chicken. A total of 360 one-day-old chickens (half males and half females) were allocated into one of 3 dietary treatments (12 replicate cages/treatment; 10 broilers/replicate cage) for 100 d of age. Experimental diets were CON: basal diet; TRT1: basal diet combined with 0.05% herbal mixture; and TRT2: basal diet combined with 0.06% GAA. All birds received a basal diet during the first 30 d, but from d 31 to 100, an experimental diet was supplied. The addition of 0.05% herbal mixture improved the average body weight gain and feed conversion ratio from d 31 to 100 as well as the overall experimental period. The cecal *Lactobacillus, Escherichia coli*, and *Salmonella* count remained consistent across all dietary treatments. Blood albumin and Superoxide Dismutase (**SOD**) levels increased in the herbal mixture supplemented diet. Additionally, there was a notable reduction in excretal NH_3 and H_2S emissions in the herbal mixture group. Furthermore, the herbal mixture group exhibited increased breast muscle weight, improved breast muscle color, improved water holding capacity, and a decrease in abdominal fat compared to the control group. Additionally, the supplementation of 0.06% GAA did not demonstrate any statistically significant impact on any evaluated parameter throughout the experiment. The results from the present investigation underscore the potential of ginseng together with artichoke extract supplementation as a viable feed additive, conferring improvements in growth performance, feed efficiency, excreta gas emission, meat quality parameters, and defense mechanism against oxidative stress in Hanhyup-3-ho chicken.

Key words: artichoke, ginseng, Hanhyup-3-ho chicken, Korean native chicken, pytogenic feed additive

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INTRODUCTION

Over the past several decades, global poultry meat production has seen a substantial rise from 9 million tons in the year 1961 to 133 million tons in 2020, where broiler chicken occupies the major portion of the production (FAO, 2021). In line with this global trend, South Korea has also experienced an upsurge in poultry meat consumption. In addition to the commercial broiler, various improved indigenous breeds are also produced for meat. Notable among these are the Hanhyup-3-ho, white-mini broiler, and Woorimatdag, which stand as hallmarks of poultry diversity in South Korea, each distinguished by its unique appearance, size, and plumage patterns (Choo et al., 2014). According to the Korean Native Chicken Association, Korean native chickens are defined as chickens that have been consistently bred for at least 7 generations. The Hanhyup-3-ho is a Korean native chicken breed for meat production (Choo and Chung, 2014). The meat derived from indigenous Korean chickens exhibits a reduced fat and higher protein concentration when contrasted with meat obtained from commercial broilers which is attractive for the consumer (Choe et al., 2010). However, the more gradual growth rate of Korean native chickens extended rearing periods, which increases their susceptibility to diseases

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in commercial production (González Ariza et al., 2021). Additionally, due to the ban on antibiotic growth promoters, poultry producers seek natural feed additives. Phyto-biotics in animal feed boosted growth, gut health, antioxidant activity, nutrient uptake, and immunity while also decreasing diarrheal incidents (Zeng et al., 2015). Different herbs, spices, and plant extracts are gaining attention for boosting growth and intestinal health without adverse effects (Li et al., 2015; Song et al., 2021).

Ginseng is a natural plant product that has been widely used in Asian herbal medicine for more than 2000 years in countries such as Japan, China, and Korea (Lü et al., 2009; Truong and Jeong, 2022). Numerous biologically active components such as ginsenosides flavonoids, triterpenoids and polysaccharides can be found in ginseng root, leaf steam and prong. Ginseng is known for its adaptogenic, antioxidant, and immune-boosting properties, which can enhance stress resistance and overall health in broilers (Lü et al., 2009; Truong and Jeong, 2022; Morshed et al., 2023). Chung and Choi (2016) found improved growth performance in broilers when 1% red ginseng was added to the broiler diet. Additionally, improved growth performance and meat quality parameters were exhibited when 300 mg/kg Ginsenoside Rg1 was supplied to a broiler diet (Song et al., 2021).

On the other hand, Artichoke (*Cynara carduncul us*) is widely used in the culinary and pharmaceutical industries (Xia et al., 2014). The main bioactive component of artichoke is cynarine, which offers antioxidant, hepatoprotective, and digestive benefits that can contribute to better nutrient utilization (Xia et al., 2014; Karimi et al., 2020). Artichoke extract have some beneficial effects such as protection the hepatotoxicity in broiler chickens (Nateghi et al., 2013), modulating the oxidative stability and meat quality of Japanese quail (Abbasi and Samadi, 2014) and enhancing the immune functions in broiler chickens (Tajodini et al., 2014).

Guanidinoacetic acid (**GAA**) is a naturally occurring creatine precursor that plays a vital role in energy metabolism and muscle function (Ostojic et al., 2018; de Souza et al., 2021). Guanidinoacetic acid has been shown to possess antioxidant properties, which can protect muscle cells from oxidative stress and support overall health in broilers (Ncho et al., 2023). adding GAA to bird diets improves performance, partly due to increased Cr levels, which enhance cellular energy and protein synthesis, such as through Arg sparing (Lawler et al., 2002). A previous study conducted by Majdeddin et al. (2020) showed that GAA supplementation at 0.12% improved feed conversion and survival capacity in broilers.

However, as local chicken consumption rises, the need for natural alternatives in antibiotic-free poultry production becomes essential. There is limited research on the impact of dietary ginseng-artichoke mixture or GAA on the growth performance of Hanhyup-3-ho chicken. Taking into consideration the potential benefits of ginseng-artichoke mixture or GAA in broiler diets, this study aims to investigate and compare their effects on growth performance, as well as nutrient digestibility, cecal microbial count, blood profile, excreta gas emission and meat quality parameters in Hanhyup-3-ho chicken.

MATERIALS AND METHODS

The feeding trial was executed at the experimental farm affiliated with Dankook University. Subsequent chemical and supplementary analyses were undertaken at the Swine Nutrition and Feed Technology Laboratory, located at Dankook University, Cheonan, South Korea. Prior to the beginning of the feeding trial, all protocols relating to animal management and care received approval from the Animal Care and Use Committee of Dankook University (Approval Number: DK-1-2230).

Source of Additive

The study evaluated 2 primary feed additives. The first one is the herbal complex, a blend of 900 mg/kg of ginseng with 900 mg/kg artichoke extract. The second additive was granular GAA, formulated at a concentration of 96% guanidinoacetic acid. Both the additives were procured from a commercial company (TVOne, Hanoi, Vietnam).

Experimental Design, Birds, and Diets

A total of 360 Hanhyup-3-ho (Korean native chicken) with an average initial body weight of 38.7 ± 0.92 g were used in this feeding trial. All the chicks were randomly allotted into 3 treatments. There were 12 replication cages (1.75 × 1.55 m^2) in each treatment where each cage had 10 birds. The experimental diets were formulated in accordance with the nutritional guidelines set forth by the National Research Council (NRC, 1994) (Table 1). Dietary treatment groups were as follows: CON, Basal diet; TRT1, Basal diet + 0.05% herbal mixture; TRT2, Basal diet + 0.06% GAA. The experiment was structured into 2 phases: Phase 1, from d 1 to 30, and Phase 2, from d 31 to 100. In the first phase, a basal diet was given to all the broilers without additives. From d 31 to 100, birds were fed with additives supplements. Throughout the experiment, both feed and water were made available to the birds *ad libitum.* Water was dispensed to the birds through nipple drinkers, with 5 nipples evenly spaced per cage. The birds were housed in a temperature-controlled room. Initially, the temperature was set to 33 ± 1°C for the first week. After this period, it was systematically reduced to 24 ± 1°C. Fluorescent lamps provided constant light for the first week (24 h/d), then reduced to 20 h daily for the rest of the feeding trial. For hygienic upkeep, the experimental room underwent weekly cleaning throughout the duration of the feeding trial.

Sampling and Measurements

Growth Measurement Body weight, and feed intake was measured by cage at d 0, 30, and 100. Average body

Table 1. Composition and nutrient levels of basal diets (%, as-fed basis).

Ingredients, %	Phase 1 (d 1−30)	Phase 2 (d 31−100)
Corn	45.42	51.46
Wheat	10.00	10.00
Soybean meal	28.59	19.14
Rapeseed meal	3.00	0.00
Distillers dried grains with solubles	2.41	5.00
Tallow	6.00	6.00
Limestone	1.06	1.31
Mono Di-calcium Phosphate	1.66	1.58
Sodium bicarbonate	0.10	0.10
DL-Methionine, 99%	0.42	0.51
Threonine 98.5%	0.16	3.26
Choline, 50%	0.10	0.10
$CuSO_4$	0.03	0.04
Salt	0.26	0.23
Lysine 50%	0.62	0.71
Tryptophan,10%	0.01	0.01
Vitamin premix[1]	0.06	0.45
Mineral premix[2]	0.10	0.10
Total	100	100
Analyzed values		
Metabolizable energy, kcal/kg	3140	3250
Dry matter, %	87.53	87.57
Crude protein, %	20.50	18.50
Crude fat, %	7.85	8.05
Crude fiber, %	2.45	2.22
Crude ash, %	5.85	5.31
Calcium, %	0.90	0.90
Available phosphorus, %	0.45	0.42
Lysine, %	1.34	1.09
Methionine, %	0.65	0.66
Cysteine, %	0.34	0.27
Threonine, %	0.91	3.79
Tryptophan, %	0.23	0.17
Methionine + cysteine, %	0.99	0.93
Digestible lysine, %	1.17	0.95
Digestible methionine, %	0.60	0.62
Digestible cysteine, %	0.26	0.21
Digestible threonine, %	0.76	3.65
Digestible tryptophan, %	0.20	0.15
Digestible methionine and cysteine, %	0.87	0.83

[1]Provided per kg of complete diet: 12 mg Cu (as $CuSO_4.5H_2O$); 85 mg Zn (as $ZnSO_4$); 8 mg Mn (as MnO_2); 0.28 mg I (as KI); 0.15 mg Se (as $Na_2SeO_3.5H_2O$).

[2]Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 mg vitaminB12.

weight gain, average feed intake, and the feed conversion ratio were calculated from the feed intake and body weight.

Cecal Microbial Count On d 100, cecal contents from 8 birds per treatment were gathered into individual, sterile culture tubes and immediately transported to the laboratory for immediate examination. In the analytical procedure, a gram from each cecal sample was amalgamated with 9 mL of sterile peptone broth (Becton, Dickinson and Company, Sparks, MD) and subjected to a 60-s vortexing. To ascertain the viable bacterial counts present, 10-fold serial dilutions (spanning from 10^{-1} to 10^{-8}) were methodically plated on distinct agar mediums: Lactobacilli MRS agar (Difco Laboratories, Detroit, MI) for lactic acid bacteria, MacConkey agar (Difco Laboratories, Detroit, MI) for coliform bacteria, and Salmonella-Shigella agar (Difco Laboratories, Detroit, MI) for *Salmonella*. Postplating, Lactobacilli agar plates were incubated anaerobically at 37°C for a 24-h duration, while both MacConkey and Salmonella-Shigella agar plates were aerobically incubated at the same temperature and time frame. Postincubation, bacterial colonies specific to *Lactobacillus, E. coli*, and *Salmonella* were enumerated, with the results articulated in the logarithm of colony-forming units per gram ($\log_{10}$ CFU/g).

Organ Weight and Meat Quality Parameters The body weight of eight birds per treatment were measured and subsequently processed at a local commercial slaughterhouse on d 100. Expert staff meticulously extracted, and weight of specific organs and tissues were measured, including the breast meat, abdominal fat, gizzard, liver, spleen, and bursa of Fabricius. The weights of these organs were then computed as a percentage relative to the bird's body weight. For further meat quality assessments, samples of the breast meat were transported to Dankook University's laboratory. Immediate evaluations were conducted on the fresh meat's surface to ascertain meat color metrics, specifically lightness (L*), redness (a*), and yellowness (b*), utilizing a Minolta Chromameter (CR-210 Minolta, Minolta Co., Ltd., Osaka, Japan). The pH of each meat sample was gauged employing a glass-electrode pH meter (WTW pH 340-A, WTH Measurement Systems Inc., Ft. Myers, FL). The water holding capacity (**WHC**) of the meat was assessed using a pressure methodology. Approximately 0.2 g of the meat sample was enveloped in a 125 mm filter paper and subjected to a pressure of 3,000 psi for a duration of 3 min. The initial meat sample area and the moisture-expressed area were demarcated and subsequently quantified with a digitizing area-line sensor (MT-10S; M.T. Precision Co. Ltd, Tokyo, Japan). Cooking loss was determined by enclosing 4 g meat portions in plastic receptacles and submerging them in a water bath set at 75°C for a half-hour. After cooling, weight of the meat samples were measured, with the weight differential expressed as a percentage to deduce cooking loss. Lastly, cumulative drip loss was evaluated by preserving 4 g meat samples in plastic bags at a temperature of 4°C. The weight of these samples was documented on d 1, 3, 5, and 7, with any weight reduction expressed as a percentage.

Blood Profile On day 100, blood samples were drawn from the cervical vein of 2 broilers in each replication. The samples were collected using K3EDTA vacuum tubes and clot activator vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). To separate the blood plasma, the samples were centrifuged at a force of 4,000 g for 15 min at a temperature of 4°C. Enzymatic activities of aspartate transaminase (**AST**) and alanine aminotransferase (**ALT**) in the plasma were evaluated using an automatic biochemistry analyzer (HITACHI 747, Hitachi, Ltd., Tokyo, Japan). For a comprehensive hematological profile, whole blood samples were subjected to analysis. Parameters such as albumin (measured in g/dL), globulin (g/dL), hemoglobin (Hb, g/dL), red blood cell (**RBC**) count, and white

Table 2. The effect of herbal mixture or guanidinoacetic acid supplementation on growth performance in Hanhyup-3-ho chicken.[1]

Items	CON	TRT1	TRT2	SEM[2]	*P* value
Average initial body weight, g	38.79	38.68	38.61	0.15	0.890
Average final body weight, g	2991[b]	3080[a]	3049[ab]	13.08	0.013
d 1−30					
Average body weight gain, g	558	560	559	5.41	0.985
Average feed intake, g	1250	1252	1249	7.64	0.989
Feed conversion ratio	2.242	2.238	2.237	0.008	0.968
d 31−100					
Average body weight gain, g	2394[b]	2482[a]	2452[ab]	14.30	0.035
Average feed intake, g	8164	8112	8129	17.80	0.501
Feed conversion ratio	3.413[a]	3.274[b]	3.317[ab]	0.022	0.033
Overall (d 1−100)					
Average body weight gain, g	2952[b]	3042[a]	3011[ab]	13.06	0.012
Average feed intake, g	9413	9364	9379	19.83	0.594
Feed conversion ratio	3.190[a]	3.081[b]	3.115[ab]	0.016	0.019
Mortality	2.5	0.8	1.7	-	-

[1]Abbreviations: CON, Basal diet; TRT1, Basal diet + 0.05% herbal mixture (ginseng with artichoke extract); TRT2, Basal diet + 0.06% guanidinoacetic acid.

[2]Standard error of means.

[a,b]means in the same row with different superscript differ significantly ($P < 0.05$). Means were calculated using 12 replicates (10 birds/replicate) per treatment.

blood cell (**WBC**) count were determined using an automated blood analyzer (ADVIA 120, Bayer, Tarrytown, NY). The serum total cholesterol level was enzymatically gauged using reagent kits (Wako Pure Chemical Industries Ltd., Tokyo, Japan). Additionally, the superoxide dismutase (**SOD**) concentration in the serum was ascertained with the aid of a commercial kit (Cayman Chemical, Ann Arbor, MI).

Excretal Gas Emission During the final week of the experiment, fresh excreta samples weighing approximately 300 g were gathered from each replication cage over 4 consecutive days. These samples were collected to assess the levels of excretal ammonia ($\mathbf{NH_3}$), hydrogen sulfide ($\mathbf{H_2S}$), total mercaptan, carbon dioxide ($\mathbf{CO_2}$), and acetic acid emission. For analysis, the excreta samples were placed in 2.6-L sealed plastic containers, with 2 containers allocated for each sample. These containers were then stored for a week at room temperature, approximately 25°C. As fermentation occurred, the concentrations of NH_3, H_2S, total methyl mercaptans, CO_2, and acetic acid in the samples were determined using a complex gas meter (MultiRAE Lite model PGM-6208, RAE Systems, NC). The measurement involved extracting a sample from the headspace air, about 2 inches above the excreta surface, which amounted to roughly 100 mL.

Statistical Analysis Data were subjected to a one-way analysis of variance (**ANOVA**) using the General Linear Model (**GLM**) procedure within SAS software (SAS Inst. Inc., Cary, NC). For variables like growth performance, cecal microbial counts, and excreta gas emission, the replication cage was designated as the primary experimental unit. Conversely, for blood profiles and meat quality attributes, individual birds were considered as experimental units. The means of various treatments were compared using the Tukey test. The results are displayed as the average along with the standard errors of means (**SEM**). $P < 0.05$ was considered as significant where $P < 0.10$ was considered as trends.

RESULTS

The effect of herbal mixture or GAA supplementation on the growth performance of Hanhyup-3-ho chicken is shown in Table 2. Average final body weight was increased ($P < 0.05$) in herbal mixture supplemented group compared to the control group. Significantly enhanced ($P < 0.05$) average body weight gain and improved ($P < 0.05$) feed conversion ratio was found in the herbal mixture supplemented during d 31 to 100 and the overall period of the experiment. Average feed intake was not altered ($P > 0.05$) through the supplementation of herbal mixture in Hanhyup-3-ho chicken diet. Additionally, GAA supplementation at 0.06% did not make any difference ($P > 0.05$) in growth performance parameters.

Supplementation of the herbal mixture at 0.05% or GAA at 0.06% failed to show any significant ($P > 0.05$) difference in cecal *Lactobacillus* count, *E. coli* and *Salmonella* count compared to the control diet in Hanhyup-3-ho chicken (Table 3).

The effects of herbal mixture or GAA on various blood parameters in Hanhyup-3-ho chicken are shown in Table 4. Serum albumin levels increased ($P < 0.05$) in the herbal mixture group. Blood SOD level was increased ($P < 0.05$) in the herbal mixture-fed birds. However, GAA supplemented diet did not show any significant ($P < 0.05$) effect on any parameters of the blood

Table 3. The effect of herbal mixture or guanidinoacetic acid supplementation on cecal microbiota in Hanhyup-3-ho chicken.[1]

Items, $\log_{10}$cfu/g	CON	TRT1	TRT2	SEM[2]	*P* value
D 100					
Lactobacillus	7.94	8.03	7.96	0.02	0.106
E. coli	6.21	6.18	6.23	0.03	0.790
Salmonella	5.35	5.28	5.32	0.05	0.965

[1]Abbreviations: CON, Basal diet; TRT1, Basal diet + 0.05% herbal mixture (ginseng with artichoke extract); TRT2, Basal diet + 0.06% guanidinoacetic acid.

[2]Standard error of means.

Table 4. The effect of herbal mixture or guanidinoacetic acid supplementation on blood profile in Hanhyup-3-ho chicken.[1]

Items	CON	TRT1	TRT2	SEM[2]	P value
D 100					
Albumin, (g/dL)	1.68[b]	1.84[a]	1.75[ab]	0.03	0.039
Globulin, (g/dL)	1.45	1.49	1.48	0.03	0.906
AST, (U/L)	190.50	192.13	192.38	3.41	0.973
ALT, (U/L)	4.63	4.50	4.38	0.17	0.847
Cholesterol, (mg/dL)	176.38	163.25	170.13	4.06	0.436
WBC, ($10^3/\mu l$)	2.13	2.04	2.0 5	0.15	0.966
RBC, ($10^6/\mu l$)	1.97	1.91	1.87	0.10	0.916
Hb, (g/dL)	9.14	9.11	8.93	0.12	0.730
SOD (ng/mL)	58.14[b]	64.69[a]	62.05[ab]	0.93	0.009

[1]Abbreviations: CON, Basal diet; TRT1, Basal diet + 0.05% herbal mixture (ginseng with artichoke extract); TRT2, Basal diet + 0.06% guanidinoacetic acid; AST, aspartate aminotransferase; ALT, alanine transaminase; WBC, white blood cells; RBC, red blood cells; Hb, Hemoglobin; SOD, superoxide dismutase.

[2]Standard error of means.

[a,b]Means in the same row with different superscript differ significantly ($P < 0.05$).

profile at the end of the trial. Moreover, no significant ($P > 0.05$) effect was found in serum globulin, AST, ALT, cholesterol, WBC, RBC, Hb in Hanhyup-3-ho chicken through dietary supplementation of herbal mixture at 0.05% or GAA at 0.06%.

The effect of supplementation of herbal mixture or GAA on different excreta gas emissions (NH_3, H_2S, Methyl mercaptans, CO_2, Acetic acid) is presented in Table 5. Herbal mixture supplementation at 0.05% decreased excretal NH_3 and H_2S emissions compared to the control diet in Hanhyup-3-ho chicken. However, 0.05% herbal mixture or 0.06% GAA failed to show significant effects in excretal methyl mercaptans, CO_2 and acetic acid emission in Hanhyup-3-ho chicken.

Table 6 shows the effect of dietary herbal mixture or GAA supplementation in organ weight and meat quality parameters in Hanhyup-3-ho chicken. Breast muscle percentage was increased ($P < 0.05$) and abdominal fat percentage was decreased ($P < 0.05$) significantly through the herbal mixture supplemented diet compared to the control diet. Other meat quality characteristics such as redness of the breast muscle color and water holding characteristics improved significantly ($P < 0.05$) in the herbal mixture supplemented group. In the case of drip loss, the herbal mixture supplemented diet tended ($P < 0.10$) to show lower drip loss in the d 5 and 7 compared to the control diet group. Guanidinoacetic acid supplementation at 0.06% has failed to show any difference ($P > 0.05$) compared to the control diet in Hanhyup-3-ho chicken.

Table 5. The effect of herbal mixture or guanidinoacetic acid supplementation on gas emission in Hanhyup-3-ho chicken.[1]

Items, ppm	CON	TRT1	TRT2	SEM[2]	P value
Finish					
NH_3	41.69[a]	38.50[b]	40.50[a]	0.40	0.010
H_2S	1.95[a]	1.81[b]	2.03[a]	0.03	0.013
Methyl mercaptans	11.19	9.94	10.44	0.46	0.559
CO_2	3700	3600	3775	66.00	0.577
Acetic acid	2.56	2.38	2.63	0.22	0.900

[1]Abbreviations: CON, Basal diet; TRT1, Basal diet + 0.05% herbal mixture (ginseng with artichoke extract); TRT2, Basal diet + 0.06% guanidinoacetic acid.

[2]Standard error of means.

[a,b]Means in the same row with different superscript differ significantly ($P < 0.05$).

Table 6. The effect of herbal mixture or guanidinoacetic acid supplementation on organ weight and meat quality in Hanhyup-3-ho chicken.[1]

Items	CON	TRT1	TRT2	SEM[2]	P value
Relative organ weight, %					
Breast muscle	10.18[b]	12.19[a]	11.32[ab]	0.37	0.029
Liver	1.83	1.80	1.89	0.04	0.712
Spleen	0.19	0.22	0.20	0.01	0.664
Abdominal fat	2.98[a]	2.26[b]	2.68[ab]	0.13	0.033
Bursa of Fabricius	0.20	0.24	0.22	0.01	0.615
Gizzard	1.57	1.49	1.40	0.07	0.693
Breast muscle color					
Lightness (L*)	53.25	53.13	54.04	0.48	0.737
Redness (a*)	10.62[b]	11.67[a]	10.50[b]	0.20	0.013
Yellowness (b*)	18.64	19.86	18.60	0.28	0.112
pH value	6.18	6.65	6.31	0.17	0.544
Cooking loss, %	20.90	18.96	22.03	0.81	0.318
WHC, %	34.30[b]	40.31[a]	35.28[b]	0.96	0.005
Drip loss, %					
d 1	1.03	1.00	1.16	0.07	0.668
d 3	2.49	1.89	2.57	0.16	0.172
d 5	3.67[a]	2.69[b]	3.52[ab]	0.20	0.087
d 7	4.58[a]	3.08[b]	4.08[ab]	0.28	0.064

[1]Abbreviations: CON, Basal diet; TRT1, Basal diet + 0.05% herbal mixture (ginseng with artichoke extract); TRT2, Basal diet + 0.06% guanidinoacetic acid; WHC, water holding capacity

[2]Standard error of means.

[a,b]Means in the same row with different superscript differ significantly ($P < 0.05$).

DISCUSSION

The Hanhyup-3-ho is a Korean native chicken specially for meat production. Traditional breeds like Hanhyup-3-ho typically exhibit a more gradual growth (González Ariza et al., 2021), which requires an extended rearing period and may enhance their disease susceptibility. Ginseng and artichoke are both acknowledged for their abundance of bioactive constituents (Tam et al., 2018; Karimi et al., 2020). Equally, GAA is esteemed in poultry feed formulations due to its pivotal role in optimizing energy metabolism (Ostojic et al., 2018; de Souza et al., 2021). In this study, we expected that incorporating an herbal mixture (ginseng blend and artichoke extract) or GAA could potentially elevate the growth performance of Hanhyup-3-ho chickens. Our observations found a significant improvement in both average body weight gain and feed conversion ratio for the group administered the herbal mixture, especially from d 31 to 100 and throughout the experimental period. This showed the efficacy of the ginseng-artichoke combination in promoting growth. Though the feed conversion ratio was a little higher than fast-growing commercial broiler. This slower growth is linked to differences in metabolic rates and energy partitioning, where a larger proportion of energy intake is allocated towards maintenance rather than rapid muscle deposition (Choo et al., 2014). The genetic traits of native chickens have evolved to prioritize survival, disease resistance, and adaptability to diverse environments over rapid growth (Moujahed and Haddad, 2013). However, there is a scarcity of research examining the combined influence of ginseng and artichoke on Hanhyup-3-ho chicken growth, making it hard to direct comparisons with previous studies. However, separate investigations have underscored the growth-promoting properties of both ginseng and artichoke in broiler chickens (Chung and Choi, 2016; Song et al., 2021; Ghasemian et al., 2022). In contrast, Ao et al., (2011) found no growth enhancements when broiler diets were supplemented with 4 g/kg of red ginseng extract. Such inconsistencies could be attributed to differences in methodological approaches and the specifics of ginseng processing (Chung et al., 2016). Our study indicates that the combined effects of ginseng and artichoke, known for their antioxidant, anti-inflammatory, immune-enhancing, liver protective, and digestive benefits (Tam et al., 2018; Ghasemian et al., 2022), play a crucial role in observed growth improvement. Ginsenoside Rg1, a primary bioactive compound in ginseng, is active in amplifying nutrient absorption and resisting pathogens, facilitated by the enhancement of intestinal morphology, supporting of tight junction integrity, and increased secretion of sIgA (Song et al., 2021). Moreover, ginseng extract has demonstrated potential in amplifying the expression of tight junction proteins, signifying its efficacy in fortifying intestinal barrier resilience, especially under heat-induced stress (Sandner et al., 2020). The observed growth improvement in this study can be attributed to the improved feed efficacy in chickens supplemented with the herbal mixture. On the other hand, the role of GAA in muscle augmentation and energizing metabolic pathways is well-documented, making it a prime candidate for enhancing broiler growth (Majdeddin et al., 2020; de Souza et al., 2021). Serving as a precursor to creatine, GAA is crucial in energy metabolism and muscle augmentation, promoting the synthesis of adenosine triphosphate the quintessential energy molecule in cells (Wyss and Kaddurah-Daouk, 2000; Brosnan and Brosnan, 2007). Such energy metabolism invariably catalyzes muscle growth, subsequently enhances growth metrics in broilers. However, in this study, GAA supplementation failed to show noticeable growth benefits in Hanhyup-3-ho chickens compared to the control group. Further research is essential to determine the most effective dosage and understand the underlying mechanisms for Hanhyup-3-ho chickens.

The supplementation of either the herbal mixture or GAA did not make any discernible changes in the cecal microbial count of Hanhyup-3-ho chicken. This finding is consistent with the observations of Song et al. (2021), who noted no alterations in chicken cecal microbiota composition upon introducing ginsenoside Rg1 to broiler diets. Similarly, Kang et al. (2016) found that the concentrations of *Salmonella* and *E. coli* in the ileum remained unaffected with the addition of red ginseng to laying hen diets. In contrast, a study by Tajudeen et al. (2023) highlighted an increase in *Lactobacillus* and a decrease in *E. coli* when laying hen diets were supplemented with 0.5% wild ginseng. Ginseng is renowned for its ginsenoside content, which is attributed with antioxidant, anti-inflammatory, and immunomodulatory activities (Tam et al., 2018; Truong and Jeong, 2022). Such properties could be essential in enhancing gut health by reducing oxidative stress and inflammation, thereby bolstering overall gut health. Enhanced energy metabolism can also indirectly be advantageous for gut microbiota, as it might optimize overall gut functioning and encourage the growth of beneficial bacteria. Artichoke, endowed with a wealth of phenolic compounds, offers antioxidant, anti-inflammatory, and antimicrobial benefits. Such attributes could potentially augment the gut environment, making it conducive to the proliferation of *Lactobacillus*. Conversely, while GAA is recognized as a precursor to creatine and boosts creatine synthesis, its influence doesn't extend directly to the gut environment (Zhao et al., 2021). The absence of marked effects on gut microbiota in our study could suggest that the provided supplementation levels of 0.05% herbal mixture or 0.06% GAA might not be potent enough to show significant improvement concerning gut health and the proliferation of advantageous bacteria.

Hanhyup-3-ho chickens exhibited an increase in serum albumin and SOD levels when supplemented with the herbal mixture in this study. Serum protein concentrations are pivotal in determining the nutritional status of broilers (Tóthová et al., 2019). The liver is the primary site of albumin synthesis, making albumin levels an informative metric for liver health assessment. A fall in serum albumin can be suggestive of potential liver ailments or compromised liver function. In contrast, maintained or elevated levels are indicative of a healthy liver (Castro and Kim, 2019; Sun et al., 2019; Mousa et al., 2023). Proper nutrient assimilation and sufficient protein intake are prerequisites for the regulation of blood protein levels. The observed elevation in serum albumin can potentially be attributed to the hepatoprotective effects of both fermented ginseng (Murthy et al., 2014) and artichoke (Ürüşan, 2023). The superoxide dismutase enzyme is important for cellular antioxidant mechanisms. An increase in SOD activity typically suggests an ameliorated cellular prowess to counter oxidative stress. A study by Kim et al. (1996) found that ginsenoside Rb2 could spur the transcription of SOD by interacting with the SOD gene's promoter region. Our results highlight elevated SOD levels after ginseng and artichoke extract supplementation. For instance, isolated ginseng supplementation has been previously documented to possess pronounced antioxidant attributes (Hong et al., 2012). The administration of ginseng and artichoke might harness their combined strengths, potentially leading to improved antioxidant defense, as manifested by the enhanced SOD activity in our study. Ginsenosides and artichoke extracts, with their renowned protective attributes against oxidative stress, can potentially modulate signaling pathways governing SOD expression (Zhao et al., 2021).

The environment of livestock farming facilities is often overloaded with harmful gases, which is a risk to the health of both the animals and the people who work there. Notably, ammonia and sulfur-based compounds stand out as the primary causes behind noxious gas emissions and environmental degradation in such settings (Ni et al., 2021). Our research reveals that when the diet of Hanhyup-3-ho chickens was supplemented with a herbal mixture, there was a noticeable decrease in the emissions of NH_3 and H_2S from their excreta. Similarly, Hassan et al. (2012) observed that the inclusion of fermented ginseng in broiler diets culminated in reduced excretal ammonia and CO_2 emissions. This aligns with previous studies that have underscored the potential of plant extracts or phytogenic feed additives in curbing volatile compounds in broiler droppings or slashing excreta ammonia gas emissions (Li et al., 2015). There is an intricate relationship between the pungent gas emissions from animal excreta and nutrient assimilation (Jeong and Kim, 2014). Increased feed efficiency typically translates to a more thorough oxidative breakdown of organic matter in the intestine. This, in turn, can lead to diminished odor and reduced levels of harmful gases in the excreta. In our study, the improved feed efficiency in Hanhyup-3-ho chickens from the herbal mixture supplemented diet suggests that the reduced excreta ammonia gas content might be attributed to enhanced digestibility.

The inclusion of herbal mixture in the Hanhyup-3-ho chicken diet resulted in enhanced breast muscle quality, higher redness in breast meat, superior water retention capacity, and a reduction in abdominal fat. Previous research has underscored the meat quality benefits of adding fermented ginseng individually to broiler feed (Ao et al., 2011). The vasodilatory properties of ginseng known to boost blood circulation can ensure optimal nutrient and oxygen delivery to muscle cells (Lee et al., 2020). Specifically, ginsenoside has been linked to increased breast muscle development in broilers (Song et al., 2021). The observed muscle improvements in chickens fed the combined ginseng-artichoke supplement can likely be attributed to the synergistic effects of these components. The enhanced redness in the breast meat of the supplemented group might stem from better muscle oxygenation, a result of the circulatory benefits of ginseng (Lee et al., 2020). The observed abdominal fat reduction aligns with the established anti-obesity properties of ginseng (Kim and Park, 2003) and the lipid-lowering effects of artichoke (Sahebkar et al., 2018). Collectively, the combined effects of ginseng and artichoke likely play a role in optimizing growth, metabolic energy utilization, and lipid regulation in the meat of Hanhyup-3-ho chicken. WHC stands as a crucial determinant of meat's juiciness and tenderness. One of the core factors affecting WHC is apoptosis, which can disrupt muscle cell structural integrity, leading to diminished WHC (Chen et al., 2022). Given that ginsenosides have been shown to counteract apoptosis in animal models (Lü et al., 2009), it is plausible that this property contributed to the observed WHC enhancement in our study.

CONCLUSIONS

Our study reveals that 0.05% herbal mixture (ginseng and artichoke extract) supplementation offers pronounced enhancements in average body weight gain, feed efficiency, blood albumin and SOD, and meat quality parameters in Hanhyup-3-ho chickens, alongside environmental benefits through reduced excreta NH_3 and H_2S emissions. Enhanced feed efficiency contributes to improving average body weight gain and lessening the organic matter responsible for noxious NH_3 and H_2S production. Moreover, the health benefit properties of ginseng and artichoke further elevate meat quality parameters. In stark contrast, GAA, at 0.06%, remained ineffective across the assessed parameters. These findings suggest the potential of the herbal mixture as an effective additive for enhancing poultry health and product quality, with possible environmental benefits in Hanhyup-3-ho chickens.

DISCLOSURES

The authors declare no direct financial interests or affiliations that could be perceived as influencing the research presented in this paper. Additionally, there are no personal relationships or interests that could be seen as influencing the research.

REFERENCES

Abbasi, F., and F. Samadi. 2014. Effect of different levels of Artichoke (*Cynara scolymus* L.) leaf powder on the performance and meat quality of Japanese quail. Poult. Sci. J. 2:95–111.

Ao, X., T. X. Zhou, H. J. Kim, S. M. Hong, and I. H. Kim. 2011. Influence of fermented red ginseng extract on broiler and laying hens. Asian-Australas. J. Anim. Sci. 24:993–1000.

Brosnan, J. T., and M. E. Brosnan. 2007. Creatine: endogenous metabolite, dietary, and therapeutic supplement. Annu. Rev. Nutr. 27:241–261.

Castro, F. L. S., and W. K. Kim. 2019. DL − methionine can be replaced partially by phyto-additive without affecting growth performance, fat metabolism, and serum biochemistry in broilers. J. Appl. Poult. Res. 28:1013–1020.

Chen, Z., T. Xing, J. Li, L. Zhang, Y. Jiang, and F. Gao. 2022. Oxidative stress impairs the meat quality of broiler by damaging mitochondrial function, affecting calcium metabolism and leading to ferroptosis. Anim. Biosci. 35:1616–1627.

Choe, J. H., K. C. Nam, S. Jung, B. N. Kim, H. J. Yun, and C. R. Jo. 2010. Differences in the quality characteristics between commercial Korean native chickens and broilers. Korean. J. Food. Sci. Anim. Resour. 30:13–19.

Choo, Y. K., and S. H. Chung. 2014. Brief review on local chicken breeds in korea with respect to growth performance and meat quality. Int. J. Poult. Sci. 13:662.

Choo, Y. K., H. J. Kwon, S. T. Oh, J. S. Um, B. G. Kim, C. W. Kang, S. K. Lee, and B. K. An. 2014. Comparison of growth performance, carcass characteristics and meat quality of Korean local chickens and silky fowl. Asian-Australas. J. Anim. Sci. 27:398–405.

Chung, T. H., and I. H. Choi. 2016. Growth performance and fatty acid profiles of broilers given diets supplemented with fermented red ginseng marc powder combined with red koji. Braz. J. Poult. Sci. 18:733–738.

Chung, S. I., S. J. Nam, M. Xu, M. Y. Kang, and S. C. Lee. 2016. Aged ginseng (Panax ginseng Meyer) reduces blood glucose levels and improves lipid metabolism in high fat diet-fed mice. Food. Sci. Biotechnol. 25:267–273.

de Souza, C., C. Eyng, A. M. Viott, A. S. de Avila, W. J. Pacheco, N. R. Junior, T. L. Kohler, K. I. Tenorio, E. H. Cirilo, and R. V. Nunes. 2021. Effect of dietary guanidinoacetic acid or nucleotides supplementation on growth performances, carcass traits, meat quality and occurrence of myopathies in broilers. Livest. Sci. 251:104659.

Food and Agriculture Organization of the United Nations (FAO). 2021. FAOSTAT database. Rome: FAO. Accessed Sep. 2023. from http://www.fao.org/faostat/en/#data/QL

Ghasemian, S. O., M. Gholami-Ahangaran, O. Pourmahdi, and A. Ahmadi-Dastgerdi. 2022. Dietary supplementation of protexin and artichoke extract for modulating growth performance and oxidative stress in broilers. Ankara. Univ. Vet. Fak. Derg. 69:281–288.

González Ariza, A., A. Arando Arbulu, F. J. Navas González, S. Nogales Baena, J. V. Delgado Bermejo, and M. E. Camacho Vallejo. 2021. The study of growth and performance in local chicken breeds and varieties: a review of methods and scientific transference. Animals 11:2492.

Hassan, M. R., H. S. Choe, and K. S. Ryu. 2012. A comparison of feeding multi-probiotics and fermented ginseng byproducts on performance, intestinal microflora and immunity of broiler chicks. Korean J. Poult. Sci. 39:253–260.

Hong, J., M. Bong, J. Park, H. Moon, S. Lee, J. Lee, and S. Hwang. 2012. Effect of feeding red ginseng marc on vital reaction in laying hens under stress task. Korean J. Poult. Sci. 39:63–70.

Kang, H. K., S. B. Park, and C. H. Kim. 2016. Effect of dietary supplementation of red ginseng by-product on laying performance, blood biochemistry, serum immunoglobulin and microbial population in laying hens. Asian-Australas. J. Anim. Sci. 29:1464–1469.

Karimi, M., F. Samadi, M. Ahmadifar, R. Nateghi, and N. Mashhadizadeh. 2020. The effects of artichoke aqueous extract on the growth performance, intestinal morphology, and blood metabolites in broiler chickens. Mod. Med. Lab. J. 3:60–68.

Kim, S. H., and K. S. Park. 2003. Effects of Panax ginseng extract on lipid metabolism in humans. Pharmacol. Res. 48:511–513.

Kim, Y. H., K. H. Park, and H. M. Rhoxy. 1996. Transcriptional activation of the Cu, Zn-superoxide dismutase gene through the AP2 site by ginsenoside Rb2 extracted from a medicinal plant, Panax ginseng. J. Biol. Chem. 271:24539–24543.

Lawler, J. M., W. S. Barnes, G. Wu, W. Song, and S. Demaree. 2002. Direct antioxidant properties of creatine. Biochem. Biophys. Res. Commun. 290:47–52.

Lee, H. J., B. M. Kim, S. H. Lee, J. T. Sohn, J. W. Choi, C. W. Cho, H. D. Hong, Y. K. Rhee, and H. J. Kim. 2020. Ginseng-induced changes to blood vessel dilation and the metabolome of rats. Nutrients 12:2238.

Li, H. L., P. Y. Zhao, Y. Lei, M. M. Hossain, and I. H. Kim. 2015. Phytoncide, phytogenic feed additive as an alternative to conventional antibiotics, improved growth performance and decreased excreta gas emission without adverse effect on meat quality in broiler chickens. Livest. Sci. 181:1–6.

Lü, J. M., Q. Yao, and C. Chen. 2009. Ginseng compounds: an update on their molecular mechanisms and medical applications. Curr. Vasc. Pharmacol. 7:293.

Majdeddin, M., U. Braun, A. Lemme, A. Golian, H. Kermanshahi, S. De Smet, and J. Michiels. 2020. Guanidinoacetic acid supplementation improves feed conversion in broilers subjected to heat stress associated with muscle creatine loading and arginine sparing. Poult. Sci. 99:4442–4453.

Morshed, M. N., J. C. Ahn, R. Mathiyalagan, E. J. Rupa, R. Akter, M. R. Karim, D. H. Jung, D. U. Yang, D. C. Yang, and S. K. Jung. 2023. Antioxidant activity of panax ginseng to regulate ROS in various chronic diseases. Appl. Sci. 13:2893.

Moujahed, A., and B. Haddad. 2013. Performance, livability, carcass yield and meat quality of Tunisian local poultry and fast-growing genotype (Arbor Acres) fed standard diet and raised outdoor access. J. Anim. Prod. Adv. 3:75–85.

Mousa, M. A., A. S. Asman, R. M. J. Ali, R. K. A. Sayed, K. A. Majrashi, K. G. Fakiha, R. A. Alhotan, and S. Selim. 2023. Impacts of dietary lysine and crude protein on performance, hepatic and renal functions, biochemical parameters, and histomorphology of small intestine, liver, and kidney in broiler chickens. Vet. Sci. 10:98.

Murthy, H. N., V. S. Dandin, and P. K. Yoeup. 2014. Hepatoprotective activity of ginsenosides from Panax ginseng adventitious roots against carbon tetrachloride treated hepatic injury in rats. J. Ethnopharmacol. 158:442–446.

Nateghi, R., F. Samadi, F. Ganji, and S. Zerehdaran. 2013. Hepatoprotective effects of *Cynara scolymus* L. extract on CCl4 induced liver injury in broiler chickens. Int. J. AgriScience. 3:678–688.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed Natl. Acad. 415 Press, Washington, DC.

Ncho, C. M., V. Gupta, and Y. H. Choi. 2023. Effects of dietary glutamine supplementation on heat-induced oxidative stress in broiler chickens: a systematic review and meta-analysis. Antioxidants 12:570.

Ni, J. Q., M. A. Erasmus, C. C. Croney, C. Li, and Y. Li. 2021. A critical review of advancement in scientific research on food animal welfare-related air pollution. J. Hazard. Mater. 408:124468.

Ostojic, S. M., M. Vranes, D. Loncar, N. Zenic, and D. Sekulic. 2018. Guanidinoacetic acid and creatine are associated with cardiometabolic risk factors in healthy men and women: a cross-sectional study. Nutrients 10:87.

Sahebkar, A., M. Pirro, M. Banach, D. P. Mikhailidis, S. L. Atkin, and A. F. G. Cicero. 2018. Lipid-lowering activity of artichoke extracts: a systematic review and meta-analysis. Crit. Rev. Food. Sci. Nutr. 58:2549–2556.

Sandner, G., A. S. Mueller, X. Zhou, V. Stadlbauer, B. Schwarzinger, C. Schwarzinger, U. Wenzel, K. Maenner, J. D. van der Klis, S. Hirtenlehner, and T. Aumiller. 2020. Ginseng extract ameliorates the negative physiological effects of heat stress by supporting heat shock response and improving intestinal barrier integrity: Evidence from studies with heat-stressed Caco-2 cells, C. elegans and growing broilers. Molecules 25:835.

Song, Z., K. Xie, Y. Zhang, Q. Xie, X. He, and H. Zhang. 2021. Effects of dietary ginsenoside rg1 supplementation on growth performance, gut health, and serum immunity in broiler chickens. Front. Nutr. 8:705279.

Sun, L., H. Yin, M. Liu, G. Xu, X. Zhou, P. Ge, H. Yang, and Y. Mao. 2019. Impaired albumin function: a novel potential indicator for liver function damage? Ann. Med. 51:333–344.

Tajodini, M., F. Samadi, S. R. Hashemi, S. Hassani, and M. Shams-Shargh. 2014. Effect of different levels of Artichoke (*Cynara scolymus* L.) powder on the performance and immune response of broiler chickens. Int. J. AgriSci. 4:66–73.

Tajudeen, H., J. Mun, S. Ha, A. Hosseindoust, S. Lee, and J. Kim. 2023. Effect of wild ginseng on the laying performance, egg quality, cytokine expression, ginsenoside concentration, and microflora quantity of laying hens. J. Anim. Sci. Technol. 65:351–364.

Tam, D. N. H., D. H. Truong, T. T. H. Nguyen, L. N. Quynh, L. Tran, H. D. Nguyen, B. E. Shamandy, T. M. H. Le, D. K. Tran, D. Sayed, V. V. Vu, S. Mizukami, K. Hirayama, and N. T. Huy. 2018. Ginsenoside Rh1: a systematic review of its pharmacological properties. Planta. Medica. 84:139–152.

Tóthová, C., E. Sesztáková, B. Bielik, and O. Nagy. 2019. Changes of total protein and protein fractions in broiler chickens during the fattening period. Vet. World. 12:598–604.

Truong, V. L., and W. S. Jeong. 2022. Red ginseng (*Panax ginseng* Meyer) oil: a comprehensive review of extraction technologies, chemical composition, health benefits, molecular mechanisms, and safety. J. Ginseng Res. 46:214–224.

Ürüşan, H. 2023. Hepatoprotective effect of Artichoke (*Cynara scolymus*) in laying hens. J. Hellenic. Vet. Med. Soc. 74:5249–5258.

Wyss, M., and R. Kaddurah-Daouk. 2000. Creatine and creatinine metabolism. Physiol. Rev. 80:1107–1213.

Xia, N., A. Pautz, U. Wollscheid, G. Reifenberg, U. Förstermann, and H. Li. 2014. Artichoke, Cynarin and Cyanidin downregulate the expression of inducible nitric oxide synthase in human coronary smooth muscle cells. Molecules 19:3654–3668.

Zeng, Z., S. Zhang, H. Wang, and X. Piao. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. J. Anim. Sci. Biotechnol. 6:7.

Zhao, W., J. Li, T. Xing, L. Zhang, and F. Gao. 2021. Effects of guanidinoacetic acid and complex antioxidant supplementation on growth performance, meat quality, and antioxidant function of broiler chickens. J. Sci. Food. Agric. 101:3961–3968.