Effects of Dietary Supplementation of Rosa roxburghii Extract on Growth Performance, Lipid Metabolism, and Hepatic Heat Shock Protein 70 Gene Expression in Wanxi White Geese under Summer High Temperature Conditions: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary Rosa roxburghii extract supplementation on growth performance, lipid metabolism, and hepatic heat shock protein 70 (HSP70) gene expression in Wanxi white geese under high summer temperature conditions. A total of 150 healthy Wanxi white geese at 28 days of age with similar body weight were randomly allocated into three groups: a control group, treatment group 1, and treatment group 2, with five replicates per group and ten geese per replicate. Geese in the control group were fed a basal diet, whereas those in treatment groups 1 and 2 were fed experimental diets supplemented with 100 and 200 mL/kg Rosa roxburghii extract, respectively. The pre-trial period lasted 7 days, followed by a 14-day formal trial period; during the experiment, the maximum temperature was 37 °C, the minimum temperature was 24 °C, and the daily average temperature was 31.6 °C. After two weeks of feeding, one goose from each replicate was slaughtered to collect blood samples and isolate the liver for determination of serum lipid metabolism indicators and hepatic HSP70 gene relative expression level. The results showed that dietary Rosa roxburghii extract supplementation had no significant effect on feed intake or average daily gain of Wanxi white geese ($P>0.05$), but supplementation at 200 mL/kg significantly reduced the feed conversion ratio ($P<0.05$). Both 100 and 200 mL/kg Rosa roxburghii extract supplementation significantly increased serum high-density lipoprotein content ($P<0.05$), while having no significant effect on serum total cholesterol and triglyceride contents ($P>0.05$). Compared with the control group, the relative expression level of HSP70 gene in the liver

was extremely significantly increased in both treatment groups 1 and 2 (P<0.01), and the relative expression level in treatment group 1 was also extremely significantly higher than that in treatment group 2 (P<0.01). It is concluded that under summer high temperature conditions, dietary supplementation of Rosa roxburghii extract in Wanxi white geese can improve feed conversion efficiency, regulate lipid metabolism, upregulate hepatic HSP70 gene expression, and exert obvious anti-stress effects, with the anti-stress effect being superior at an inclusion level of 100 mL/kg compared to 200 mL/kg.

Full Text

Effects of Rosa roxburghii Tratt. Extract on Growth Performance, Lipid Metabolism and Heat Shock Protein 70 Gene Expression in Liver of Wanxi White Geese under High Temperature in Summer

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Abstract

This study investigated the effects of dietary Rosa roxburghii Tratt. (RRT) extract supplementation on growth performance, lipid metabolism, and heat shock protein 70 (HSP70) gene expression in the liver of Wanxi white geese under high summer temperatures. One hundred fifty 28-day-old healthy Wanxi white geese with similar body weight were randomly allocated into three groups: a control group, trial group 1, and trial group 2. Each group consisted of five replicates with ten geese per replicate. Geese in the control group received a basal diet, while those in trial groups 1 and 2 received experimental diets supplemented with 100 and 200 mL/kg of RRT extract in the basal diet, respectively. The pre-test period lasted 7 days, followed by a 14-day experimental period. During the trial, the maximum temperature reached 37 °C, the minimum was 24 °C, and the average daily temperature was 31.6 °C. After two weeks of feeding, one goose from each replicate was selected for slaughter; blood samples were collected and livers were harvested to determine serum lipid metabolism indices and the relative expression level of the HSP70 gene in liver tissue. The results showed that RRT extract supplementation had no significant effect on average daily feed intake or average daily gain ($P > 0.05$). However, supplementation with 200 mL/kg RRT extract significantly reduced the feed-to-gain ratio ($P < 0.05$). Both 100 and 200 mL/kg RRT extract supplementation significantly increased serum high-density lipoprotein content ($P < 0.05$) but did not signifi-

cantly affect serum total cholesterol or triglyceride levels ($P > 0.05$). Compared with the control group, the relative expression level of the HSP70 gene in the liver was extremely significantly increased in both trial groups ($P < 0.01$), with trial group 1 showing an extremely significantly higher expression level than trial group 2 ($P < 0.01$). These findings indicate that under high summer temperature conditions, dietary RRT extract supplementation in Wanxi white geese can improve feed conversion efficiency, regulate lipid metabolism, and upregulate hepatic HSP70 gene expression, demonstrating notable anti-stress effects. The anti-stress efficacy was superior at the supplementation level of 100 mL/kg compared to 200 mL/kg.

Keywords: Wanxi white geese; Rosa roxburghii Tratt. extract; growth performance; lipid metabolism; HSP70 gene expression

Introduction

Rosa roxburghii Tratt. (RRT) is a wild medicinal plant of the Rosaceae family primarily distributed in southern China. Research has demonstrated that RRT fruit can enhance immunity and exhibits inhibitory effects against various diseases, including atherosclerosis, gastric cancer, and ovarian cancer. The fruit is rich in vitamins, trace elements, amino acids, polysaccharides, and other bioactive compounds. Notably, its vitamin C content reaches 664–2,601 mg per 100 g of fresh fruit, substantially higher than that of other fruits and vegetables, suggesting potential anti-stress properties.

Heat shock proteins (HSPs), also known as stress proteins, are a highly conserved family of proteins produced in response to various environmental stimuli such as heat and cold. During stress conditions, HSPs participate in cellular protection, damage repair, and thermotolerance processes, thereby safeguarding cellular activities. Among the various HSPs, HSP70 is the primary stress protein. During heat stress, increased HSP70 expression enhances cellular thermotolerance and protects organisms from damage, making HSP70 expression a potential biomarker for environmental stress.

Currently, research on RRT extract application in animals remains limited, particularly regarding its effects on goose growth performance and serum lipid content. Furthermore, whether RRT extract can mitigate stress responses by regulating HSP70 gene expression has not been reported. Therefore, this study aimed to investigate the effects of dietary RRT extract supplementation on growth performance, serum lipid content, and hepatic HSP70 gene expression in Wanxi white geese under high summer temperatures, providing data to support the application of RRT extract in animal production.

Materials and Methods

1.1 Experimental Material

RRT samples were purchased from Kaifeng Jinweikang Wild Plant Development Co., Ltd. Dried RRT fruit was ground into powder, wrapped in gauze (200 g per package), and soaked in distilled water at 45 °C for 2 hours. Ultrasonic extraction was performed at 50 °C with a 1:10 material-to-liquid ratio (40% ethanol) at 40 kHz frequency and 200 W power for 40 minutes. The extract was then concentrated at 60 °C under negative pressure (-0.08 to -0.05 MPa) to approximately 55% of the initial volume and stored at 4 °C for later use. A portion of the extract was analyzed for vitamin C, polyphenol, and flavonoid content.

Vitamin C content in the RRT extract was determined by high-performance liquid chromatography. A small amount of concentrated extract was filtered through a Buchner funnel, and 1 mL of the filtrate was accurately measured into a 10 mL brown volumetric flask, diluted with 1 g/L metaphosphoric acid solution, sonicated for 5 minutes, and filtered through a 0.45 m membrane before analysis using the method described by Wang et al. Flavonoid and polyphenol contents were measured by colorimetric methods.

1.2 Experimental Design and Animals

One hundred fifty 28-day-old healthy Wanxi white geese with similar body weight were randomly divided into three groups: control, trial group 1, and trial group 2. Each group comprised five replicates with ten geese per replicate. The control group received a basal diet, while trial groups 1 and 2 received experimental diets supplemented with 100 and 200 mL/kg RRT extract in the basal diet, respectively.

1.3 Experimental Diets

The basal diet was formulated according to NRC (1994) goose feeding standards and actual production practices in China. Experimental diets were prepared by adding 100 and 200 mL/kg of RRT extract to the basal diet, maintaining identical nutrient levels across all diets. The composition and nutrient levels of the basal diet are presented in Table 1.

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

Ingredients: Corn, Soybean meal, Limestone, $CaHPO_4$, Premix
Nutrient levels: Metabolizable energy (ME) (MJ/kg), Crude protein (CP), Calcium (Ca), Available phosphorus (AP), Lysine (Lys), Methionine (Met)

Premix provided per kilogram of diet: NaCl 3 g, 50% choline 1 g, Lysine 1 g, VA 10,000 IU, VD_3 2,500 IU, VE 50 mg, VK 4.5 mg, VB_{12} 0.28 mg, VB_1 8.75 mg, VB_6 1.7 mg, Niacin 35 mg, Pantothenic acid 13 mg, Folic acid 2 mg, Biotin 0.047 mg, Cu 150 mg, Zn 100 mg, Fe 130 mg, Mn 30 mg, I 0.35 mg, Se 0.25 mg.

All indices were calculated values.

1.4 Animal Management

The experiment was conducted at the Animal Research Base of Henan University of Animal Husbandry and Economy from July 12 to August 2, 2015, lasting 21 days total (7-day pre-test period and 14-day experimental period). During the trial, the maximum temperature was 37 °C, the minimum was 24 °C, and the average daily temperature was 31.6 °C. Geese were raised on the floor with ad libitum access to feed and water. Routine management practices were followed. Daily temperature was recorded, and feed intake was recorded per replicate. Flock health status was monitored morning and evening. At the end of the experiment, one goose per replicate was selected for slaughter; blood samples were collected and livers were harvested for serum index analysis and HSP70 gene expression measurement.

1.5 Sample Collection and Analysis

1.5.1 Growth Performance Measurement Daily feed intake per replicate was accurately recorded to calculate average daily feed intake (ADFI) during the experimental period. Body weight was measured at the beginning and end of the experiment after overnight fasting to calculate average daily gain (ADG) and feed-to-gain ratio (F/G).

1.5.2 Serum Lipid Metabolism Indices Measurement At the end of the experiment, one goose per replicate was selected, weighed, and slaughtered for blood collection (10 mL). Blood samples were allowed to clot and then centrifuged at 3,000 r/min for 15 minutes. Serum was collected in EP tubes and stored at -20 °C for subsequent analysis. Serum alanine aminotransferase (ALT) activity and high-density lipoprotein (HDL), total cholesterol (TC), and triglyceride (TG) contents were measured by the Clinical Laboratory of Zhengzhou Fifth People' s Hospital.

1.5.3 Determination of Relative HSP70 Gene Expression in Liver Liver samples (30 mg) were collected from each slaughtered goose, fully ground in liquid nitrogen, and placed in 1.5 mL EP tubes. Total RNA was extracted using an RNA extraction kit (SK1312, Shanghai Sangon Biotech Co., Ltd.) according to the manufacturer' s instructions. The extracted total RNA served as a template for reverse transcription (RT) using a cDNA synthesis kit (SK2445, Shanghai Sangon Biotech Co., Ltd.). The RT reaction mixture (20 L total volume) contained: total RNA 5 L, OligodT primer 1 L, RNase inhibitor 1.0 L (20 U/ L), dNTP Mixture 2 L (10 mmol/L), 5×RT buffer 4 L, reverse transcriptase 2 L (10 U/ L), and RNase-free deionized water 5 L. RT was performed in a Mastercycler Gradient PCR instrument using the following program: 70 °C for 5 min, ice bath for 10 s, 37 °C for 5 min, 42 °C for 60 min, and 70 °C for 10 min. Quantitative PCR was performed immediately after RT completion.

Primers for the target gene HSP70 and the reference gene β-actin were designed and synthesized by Shanghai Sangon Biotech Co., Ltd. The HSP70 primer sequences were: forward, 5' -AGCAGCTATCCTCATGGGAGA-3' ; reverse, 5' -TGGTGGGAATGGTGGTGTTA-3' . The β-actin primer sequences were: forward, 5' -AGTGTCTTTTTGTATCTTCCGCC-3' ; reverse, 5' -CCACATACTGGCACTTTACTCCTA-3' . The quantitative PCR reaction mixture (20 L total volume) contained: cDNA 2 L, SYBR Green Realtime PCR Master Mix 10 L, forward and reverse primers (10 mol/L) each 1 L, and 0.1% DEPC-treated water 6 L. The qPCR program was: 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, and final extension at 70 °C for 5 min. Ct values for both HSP70 and β-actin were obtained, and relative HSP70 gene expression was calculated using the 2^(-ΔΔCt) method.

1.6 Statistical Analysis

Data were analyzed using SPSS 17.0 statistical software. One-way ANOVA was performed, followed by LSD multiple comparison tests. Results are expressed as means ± standard deviation.

Results

2.1 Active Component Content of RRT Extract

The contents of vitamin C, polyphenols, and flavonoids in RRT extract are presented in Table 2. The data indicate that RRT extract is rich in vitamin C, reaching 90.9 mg/g DM.

Table 2. Contents of active ingredients of RRT extract (n=5), mg/g DM

Item	Vitamin C	Polyphenol	Flavonoid
Content	90.92$±6.24|39.54±1.79|41.05±$3.87		

2.2 Effects of RRT Extract on Growth Performance of Wanxi White Geese

The effects of RRT extract on growth performance are shown in Table 3. Dietary supplementation with 100 and 200 mL/kg RRT extract did not significantly affect average daily feed intake or average daily gain ($P > 0.05$). However, supplementation with 200 mL/kg RRT extract significantly reduced the feed-to-gain ratio ($P < 0.05$).

Table 3. Effects of RRT extract on growth performance of Wanxi white geese

Items	Control group	Trial group 1	Trial group 2
Initial BW (kg)	1.08$±0.08	1.11±0.04	1.06±0.03 || $FinalBW(kg)$ | 1.89±0.09	2.07±0.05	1.98±0.04 || $ADFI(g)$ | 105.74

In the same row, values with different small letter superscripts indicate significant difference (P < 0.05), and different capital letter superscripts indicate extremely significant difference (P < 0.01). The same as below.

2.3 Effects of RRT Extract on Serum Parameters of Wanxi White Geese

The effects of RRT extract on serum parameters are presented in Table 4. Compared with the control group, both trial groups exhibited significantly increased serum high-density lipoprotein content (P < 0.05). However, serum total cholesterol and triglyceride levels remained unchanged (P > 0.05), suggesting that RRT extract may be involved in lipid metabolism regulation. Additionally, no significant differences in serum alanine aminotransferase activity were observed among groups (P > 0.05).

Table 4. Effects of RRT extract on serum parameters of Wanxi white geese

Items	Control group	Trial group 1	Trial group 2
ALT (U/L)	13.25$±2.40	13.00±0.71	14.25±0.95 || $HDL(mmol/L)$ | 1.21±0.07^{a}	1.55±0.16^{b}	1.58±0.09^{b} || $TC(mmo$

2.4 Effects of RRT Extract on Relative HSP70 Gene Expression in Liver of Wanxi White Geese

As shown in Table 5, the relative expression level of HSP70 gene in the liver of trial group 2 was extremely significantly lower than that of trial group 1 (P < 0.01). However, both trial groups exhibited extremely significantly higher HSP70 gene expression compared with the control group (P < 0.01), indicating enhanced anti-stress capacity, particularly against heat stress, and demonstrating that RRT extract has significant anti-stress effects in geese.

Table 5. Effects of RRT extract on relative expression level of HSP70 gene in liver of Wanxi white geese

Item	Control group	Trial group 1	Trial group 2
Relative expression level	1.11$±0.10^{A}	4.06±0.54^{B}	1.95±$0.29

Discussion

3.1 Effects of RRT Extract on Growth Performance of Wanxi White Geese

Research on the effects of RRT extract on animal growth performance is limited and yields inconsistent results. Zhang reported that oral administration of 100 mg/kg RRT extract for 4 weeks had no significant effect on feed intake or average daily gain in rats. Yang et al. found that dietary supplementation with 100 and 150 mL/kg RRT extract significantly improved average daily gain and average daily feed intake in broiler chickens, possibly due to active components such as polyphenols and organic acids in the crude extract promoting digestive fluid secretion and appetite, thereby enhancing digestive capacity. In the current study, dietary supplementation with 100 mL/kg RRT extract showed a trend toward increased average daily feed intake, though the difference was not statistically significant. These discrepancies may be attributed to differences in animal species, as well as the fact that the current trial was conducted in July under hot weather conditions that could suppress feed intake. Additionally, the relatively short 2-week experimental duration may have influenced growth performance outcomes. The significant reduction in feed-to-gain ratio observed with 200 mL/kg RRT extract supplementation may be explained by the relatively lower average daily feed intake in this group compared with the 100 mL/kg group, while average daily gain did not differ significantly between the two groups. Further research is needed to clarify the effects of RRT extract on animal growth performance.

3.2 Effects of RRT Extract on Lipid Metabolism of Wanxi White Geese

Dai et al. reported that RRT juice regulates lipid metabolism, finding that 4 weeks of RRT juice supplementation in quails significantly increased plasma total cholesterol content and moderately increased triglyceride levels, while showing a trend toward decreased plasma high-density lipoprotein. The authors suggested that lipid metabolism regulation by RRT may be based on its rich flavonoid content (60 mg/g). Xie et al. also demonstrated that dietary flavonoid supplementation at various levels significantly reduced serum total cholesterol and triglyceride contents while significantly increasing serum high-density lipoprotein in broiler chickens. Zhang analyzed the composition and content of flavonoids in RRT and found that oral administration of 100 mg/kg RRT extract for 4 weeks significantly reduced serum triglyceride levels in normal rats without significantly affecting other serum biochemical indices. In the present study, RRT extract contained 41.05 mg/g DM of flavonoids and significantly increased serum high-density lipoprotein content. High-density lipoprotein transports cholesterol from peripheral tissues to the liver for catabolism and excretion, thereby reducing serum cholesterol levels and inhibiting atherosclerosis. This mechanism may underlie the anti-atherosclerotic effects of RRT.

3.3 Effects of RRT Extract on Hepatic HSP70 Gene Expression in Wanxi White Geese

HSP70 is an anti-stress protein that is rapidly synthesized in cells under stress conditions to resist stress-induced tissue damage and enhance thermotolerance, thereby protecting the organism. To our knowledge, no previous studies have investigated the effects of RRT extract on HSP70 gene expression in goose liver. Recent research indicates that certain anti-heat-stress traditional Chinese medicines can improve stress resistance by upregulating HSP70 gene expression. RRT is a medicinal plant with extremely high vitamin C content, reaching 664–2,601 mg per 100 g of fresh fruit, substantially higher than other fruits and vegetables, suggesting potential anti-stress properties. Joseph et al. discussed that vitamin C can alleviate heat stress in broiler chickens during hot summers. Han et al. demonstrated that vitamin C supplementation in drinking water upregulated HSP70 expression and mitigated heat stress in mice. The current study also showed that dietary supplementation with 100 and 200 mL/kg RRT extract extremely significantly increased the relative expression level of the HSP70 gene in goose liver, thereby alleviating heat stress induced by high summer temperatures. However, the HSP70 expression level in the 200 mL/kg group, while extremely significantly higher than the control, was extremely significantly lower than in the 100 mL/kg group. This may be because excessive RRT extract supplementation exhibits some toxicity, and higher supplementation levels result in greater vitamin C intake. Excessively high doses of vitamin C can inhibit HSP70 gene expression. Further research is warranted to elucidate the effects of RRT extract on anti-heat-stress gene expression.

In summary, dietary RRT extract supplementation in Wanxi white geese under high summer temperature conditions can improve feed conversion efficiency, regulate lipid metabolism, and upregulate the expression of the anti-stress gene HSP70, thereby demonstrating anti-heat-stress effects. The anti-stress efficacy was superior at a supplementation level of 100 mL/kg compared to 200 mL/kg.

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Note: Figure translations are in progress. See original paper for figures.