

Effects of dietary nicotinic acid supplementation on meat quality, carcass characteristics, lipid metabolism, and tibia parameters of Wulong geese

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ABSTRACT The purpose of this study was to evaluate the effects of nicotinic acid (NA) supplementation on the meat quality, carcass characteristics, lipid metabolism, and tibia parameters in Wulong geese. A total of 360 twenty-nine-day-old Wulong geese were randomly divided into 6 treatments, and each treatment included 6 pens with 10 birds per pen. Birds were fed a basal diet supplemented with 0, 20, 40, 60, 80, or 100 mg/kg NA for 12 wk. Dietary NA supplementation linearly decreased L* value and increased pH and water-holding capacity in the breast muscle ($P < 0.05$). Increasing NA levels linearly and quadratically decreased shear force of breast muscle ($P < 0.001$). Dietary NA supplementation linearly reduced the thickness of subcutaneous fat plus the skin and percentage of abdominal fat, and enhanced the width of intermuscular fat band ($P < 0.001$). Dietary NA addition linearly and quadratically increased intramuscular fat (IMF) content ($P \leq 0.001$). Increasing NA levels decreased serum total cholesterol and low-density

lipoprotein cholesterol levels and increased serum lipase activity and hepatic mRNA expression of lipoprotein lipase in a linear manner ($P < 0.05$). There were linear and quadratic effects in serum triglycerides and high-density lipoprotein cholesterol (HDL-C) levels and malate dehydrogenase activity with the NA addition ($P < 0.05$). Feeding the NA-supplemented-diets linearly increased tibia length, circumference, fat-free dry weight, and ash content ($P < 0.001$). There were linear and quadratic increases in Ca and P contents with the NA supplementation ($P < 0.05$). According to the quadratic regression analyses fitted to shear force, IMF content, serum triglycerides and HDL-C levels, and tibial Ca and P contents, the optimal dietary NA supplementation was 80 to 90 mg/kg. In conclusion, NA addition enhanced meat quality and IMF content, regulated lipid metabolism, and increased tibia quality of Wulong geese. The dosage of 80 mg/kg NA in Wulong geese aged 5 to 16 wk was recommended.

Key words: nicotinic acid, goose, meat quality, lipid metabolism, tibia parameters

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INTRODUCTION

Nicotinic acid (NA), also known as niacin, is one of the naturally occurring B3 vitamins. Other forms of vitamin B3 include nicotinamide (Makarov et al., 2019). Vitamin B3 acting as the precursor of NAD⁺/NADH and NADP⁺/NADPH, participates in many biochemical processes, including lipid metabolism, tissue oxidation, glycolysis, and respiratory functions (MacKay et al., 2012; Srivastava, 2016; Makarov et al., 2019). For lipid metabolism, NA reduces the production of triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol, and enhances the level of high-density lipoprotein cholesterol (HDL-C) in liver by inhibiting the expression of diacylglycerol

acyltransferase 2 (DGAT2) gene (Ganji et al., 2004; Hu et al., 2012; Kashyap et al., 2019).

NA has been used in attempt to improve the quality of meat products. Study showed that NA supplementation at 550 mg/kg improved the pork quality, as measured by drip loss, pH, and color (Real et al., 2002). Jiang et al. (2011) have demonstrated that the diet supplemented with 60 mg/kg NA increased daily gain and reduced drip loss in chicken. Moreover, Jiang et al. (2014) also found that supplementation of 120 mg/kg NA reduced the percentage of abdominal fat (PAF) and the thickness of subcutaneous fat plus the skin (SFS). Recent studies have shown that 1,000 mg/kg NA-supplemented diet increased the intramuscular fat (IMF) content of Chinese steers (Yang et al., 2016).

NA deficiency is easy to cause leg diseases in poultry (Battig et al., 1953; Serafin, 1981). Previous studies showed that a NA deficiency was responsible for perosis in goslings, and this deficiency was entirely prevented by the addition of 40 mg/kg NA (Battig et al., 1953). Serafin (1981) reported that the addition of 30 mg/kg of NA

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to the diet prevented bowed legs and perosis and promoted rapid growth of goslings. However, high dietary niacin intake may be associated with osteoporosis in human (Carbone et al., 2019) and decreased tibia strength and weights in chicks (Johnson et al., 1992). The difference of the roles of NA on bone remains further studying.

The Wulong goose, also called Huoyan goose, is an important local goose species widely distributed in the east and northeast of China. Wulong goose has the characteristics of small body size, excellent egg laying performance, fresh meat quality, high early growth rate, and good resistance with crude feed (Miao et al., 2019). However, few studies have reported the effects of dietary NA supplementation on Wulong geese. The objective of the present research was to investigate the effects of NA on the meat quality, carcass characteristics, lipid metabolism, and tibia parameters of Wulong geese.

MATERIALS AND METHODS

Experimental Design and Animals

This study was approved by the Animal Care and Use Committee of Qingdao Agricultural University. Three hundred and sixty 29-day-old Wulong geese with an average initial body weight of 1.33 ± 0.02 kg were randomly divided into 6 treatments. Each treatment consisted of 6 pens of 10 birds each (half male and half female). A control group (CTR) was fed a corn-soybean meal basal diet, and 5 experimental groups, NA20, NA40, NA60, NA80, and NA100, were fed the experimental diets with 20, 40, 60, 80, and 100 mg NA being added to basal diet per kg feed for 12 wk, respectively. The analyzed level of NA in the basal diet was 23.48 mg/kg. NA was purchased from Qingdao Puxing Biotechnology Co., Ltd. (Qingdao, China) with an effective content of 99%. The basal diets were formulated based on the NRC (1994) requirements. The composition and nutritional levels of the basal experimental diet are shown in Table 1. All geese were raised on floor. The water and feed were supplied ad libitum. The experiment lasted 12 wk.

Sample Collection

At the end of the experiment, one bird close to the average weight was randomly selected from each pen and weighed after 12 h of feed deprivation. Blood samples were collected from the wing veins and then the geese were slaughtered by jugular vein exsanguination. The blood samples were centrifuged at $3,000 \times g$ for 10 min at 4°C and then the serum was collected and stored at -20°C for lipid metabolism analysis. Liver samples were collected, rapidly frozen in liquid nitrogen and stored at -80°C for mRNA analysis. Moreover, the cranial side of the pectoralis major was cut and stored at 4°C for meat quality measurements. The right tibiae were collected and frozen at -20°C prior to analysis.

Table 1. Composition and nutrient levels of basal diet (air-dry basis).

| Ingredient | Composition (%) | Nutrient ² | Level |
|--|-----------------|------------------------------|-------|
| Corn | 61.97 | ME (MJ/kg) | 11.29 |
| Soybean meal (44% CP) | 22.00 | CP (%) | 16.30 |
| Fish meal | 1.50 | CF (%) | 4.98 |
| Middling | 4.00 | Ca (%) | 0.70 |
| Corn stover | 8.00 | Nonphytate phosphorus (%) | 0.45 |
| CaHPO ₄ | 0.78 | Lys (%) | 0.82 |
| Limestone | 0.95 | Met (%) | 0.26 |
| NaCl | 0.30 | Cys (%) | 0.27 |
| Vitamin and trace mineral premix ¹ | 0.50 | Thr (%) | 0.58 |
| Total | 100.00 | Nicotinic acid (mg/kg) | 23.48 |

¹Vitamin and trace mineral premix supplied the following per kilogram of diet: Vitamin A 1,500 IU, vitamin D₃ 200 IU, vitamin E 12.5 mg, vitamin K₃ 1.5 mg, vitamin B₁ 2.2 mg, vitamin B₂ 5.0 mg, pantothenate 15 mg, vitamin B₆ 2 mg, biotin 0.2 mg, folic acid 0.5 mg, choline 1000 mg, Fe 85 mg, Cu 5 mg, Mn 80 mg, Zn 80 mg, I 0.42 mg, Co 2.5 mg.

²CP, Ca and nicotinic acid were analyzed values; others were calculated values.

Meat Quality Measurements

At 24-h postmortem, the cranial side of the pectoralis major was selected to measure meat quality (color, pH, shear force, drip loss, and water-holding capacity). The muscle color parameters, lightness (L*), redness (a*), and yellowness (b*) were measured using a handheld colorimeter (Konica Minolta CR-400, Tokyo, Japan). An aperture size of 10 mm, illuminant D65, and 2° observer angle were used. The muscle pH value was measured with an acidometer (testo 205, Lenzkirch, Germany). The shear force was measured according to Shen et al. (2019) with a Warner-Bratzler shear device (Warner-Bratzler meat shear, Bodine Electric Company, Chicago, IL) attached to a texture analyzer (TA-XT2i, Stable Micro Systems, UK). In brief, fillets were cooked in plastic bags in a water bath to an internal temperature of 70°C, as measured by a Thermo Plus thermocouple with a 10-cm needle temperature probe (Thermo Works Model 221-071, UT). The cooked fillets were cooled to 4°C and then dried with paper towels. Three samples per breast fillet were sheared in a direction perpendicular to the muscle fibers with a crosshead speed of 1 mm/s. The peak force was recorded when cutting the samples and averaged to obtain a single shear force value for each breast muscle. The drip loss was determined as described by Córdova-Noboa et al. (2018) with some modifications. Briefly, a $3 \times 2 \times 1$ cm³ cut of breast meat from the same location was weighed and hung on a hook. Then this sample was suspended in a sealed plastic bag and stored at 4°C for 24 h. The meat was then gently wiped and reweighed. The drip loss was calculated as a percentage of the weight difference relative to the initial sample weight. The water-holding capacity was measured using a filter press method as described previously (Aristides et al., 2018). Approximately 2 g of meat samples were weighed and placed between 2 filter papers. Then they were kept under

pressure of 10 kg for 5 min and weighed again. The water-holding capacity was expressed as a percentage of final sample weight with respect to the initial weight.

Carcass Characteristics

The thickness of SFS from a cross incision at the dorsal base of the pygostyle and the width of intermuscular fat band (IFB) at the caudal end of the sternum were determined using a vernier caliper after dressing. The width of IFB was determined by the average values from the 3 different locations (the 2 ends and middle of the IFB). The ratio of abdominal fat mass with respect to body weight was calculated as PAF. The content of IMF of pectoralis major muscle, trimmed of visible fat surrounding and lying around muscles, was determined by Soxhlet extraction (Jiang et al., 2011) and expressed as the weight percentage of dry muscle tissue.

Lipid Metabolism

Serum total cholesterol (TCH), TG, HDL-C, and LDL-C levels were assayed by enzymic procedures using an automatic biochemical analyzer (Hitachi 7170; Hitachi, Tokyo, Japan) and commercial assay kits (Nanjing Jiancheng Biotechnology Institution, Nanjing, China) according to the manufacturer's manuals. The assays are briefly described as follows: serum TCH and TG were determined by cholesterol oxidase-peroxidase-amidopyrine (CHO-PAP) method (Allain et al., 1974) and glycerol phosphate oxidase-peroxidase-amidopyrine (GPO-PAP) method (Koditschek and Umbreit, 1969), respectively. For HDL-C and LDL-C assays, HDL or LDL was separated by specific detergent for disruption and elimination of other lipoproteins. Then cholesterol was released by cholesterol esterase and H₂O₂ was produced by cholesterol oxidase. The level of H₂O₂, proportional to the level of cholesterol, was measured by colorimetric method in the presence of 4-aminoantipyrine and N,N-bis (4-sulfobutyl) -m-toluidine (Nakamura, 1997; Arranz-Peña et al., 1998).

Serum malate dehydrogenase (MDH) activity was measured using a MDH assay kit (Nanjing Jiancheng Biotechnology Institution, Nanjing, China). The decrease in absorbance at 340 nm due to the oxidation of NADH was assayed to determine the MDH activity. One unit of MDH activity was defined as the amount of enzyme required to oxidize 1 μmol NADH per min under the specified conditions (Yin et al., 2012). The serum lipase activity was determined spectrophotometrically

at 420 nm according to a lipase kit (Nanjing Jiancheng Biotechnology Institution, Nanjing, China). One unit of lipase activity was defined as the amount of enzyme needed to catalyze the release of 1 μmol of fatty acids in 1 min at 37°C (Li et al., 2017).

Lipoprotein Lipase mRNA Expression in Liver

The liver tissue samples were collected and immediately frozen in liquid nitrogen, and stored at -80°C until analysis. Total RNA was extracted using Trizol reagent (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. Gel electrophoresis was used to detect the RNA integrity and purity. The concentration of RNA was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). Total RNA (1 μg) was used to generate cDNA in a final volume of 20 μL using the PrimeScript RT reagent kit with cDNA eraser (Takara, Dalian, China). The specific primer sequences of Lipoprotein lipase (LPL) and β-actin gene (Table 2) were designed with Primer Express 2.0 software (Applied Biosystems, Life Technologies, Carlsbad, CA) and verified. Quantitative PCR was carried out in a 20-μL reaction volume using the SYBR Premix Ex Taq kit (Takara, Dalian, China). The mixtures were amplified using an ABI 7500 Real-Time PCR Detection System (Applied Biosystems, Life Technologies). A mixture of the CTR group samples served as the calibrator sample included on all plates for inter-plate calibration. All samples were run in triplicate. The relative mRNA expression levels of LPL gene were calculated based on the β-actin gene using the 2^{-ΔΔCT} method (Livak and Schmittgen, 2001).

Tibia Parameters

The right tibiae were removed and cleaned of soft tissue. The tibia length and circumference were measured by a vernier caliper. Tibia length was the distance from the proximal section to the position between the third and fourth toes. Tibia circumference was the perimeter of the middle part of tibia. After boiled for 30 min with deionized water, the tibiae were cleaned of cartilage and then oven-dried at 75°C for 48 h. Subsequently, the bones were defatted using sequential 2-wk Soxhlet extractions with diethyl ether. Tibial fat-free dry weight was obtained by drying the defatted bone in an oven at 105°C for 24 h. Tibial ash content was measured according to GB/T 6438-2007 (People's Republic of China

Table 2. Primer sequences of LPL and β-actin genes.

| Gene name | Accession number | Primer sequence ¹ (5' to 3') | Product size (bp) | Efficiency (%) |
|-----------|------------------|--|-------------------|----------------|
| LPL | XM_013188252.1 | F: GGACGGTGACAGGCATGTATGA R: CAGCAGGATCCAGCCCAGTAAT | 312 | 108.6 |
| β-actin | XM_013194160.1 | F: AGACCACCTTCAACTCCATCAT R: ATCTCCTTCTGCATCCTGTC | 124 | 101.3 |

Abbreviation: LPL, lipoprotein lipase.

¹F, forward; R, reverse.

National Standard, 2007). Tibial calcium (Ca) content was determined using permanganate titration method according to GB/T 6436-2018 (People's Republic of China National Standard, 2018a). Tibial total phosphorus (P) content was colorimetrically measured using the vanadate-molybdate method according to GB/T 6437-2018 (People's Republic of China National Standard, 2018b). The ash, Ca, and P contents were expressed on basis of tibial dry weight.

Statistical Analysis

Data were analyzed by one-way ANOVA procedure using SPSS statistical software (version 20.0, SPSS, Inc., Chicago, IL) appropriate for a randomized complete design. Duncan's multiple range test was used to determine significant differences among the treatment means. Polynomial contrasts were used to test the linear and quadratic effects of NA supplemented levels. Quadratic regressions ($Y = aX^2 + bX + c$) were fitted to the responses of the dependent variables to dietary NA supplemented levels. The extremum response for NA was defined as $NA = -b/(2 \times a)$. Pen was the experimental unit. The data are presented as means and pooled SEM. Statistical significance was declared at $P < 0.05$.

RESULTS

Meat Quality

The data of meat quality are shown in Table 3. Geese fed with NA supplemented-diets linearly decreased the L^* value of breast muscle ($P < 0.001$), and the minima occurred in the NA80 and NA100 groups. There were no differences in a^* and b^* values under any NA dosage ($P > 0.05$). Feeding diets supplemented with NA linearly increased the pH value of breast muscle ($P = 0.006$), and geese in the NA80 group had the highest pH value among all groups. Shear force was decreased in a linear ($P < 0.001$) and quadratic ($P < 0.001$) manner as dietary NA supplementation increased, and groups NA60 and NA80 had the lowest shear force. There was no difference in drip loss among all groups ($P > 0.05$). The

Table 4. Effects of nicotinic acid on carcass characteristics of Wulong geese ($n = 6$).

| Treatment ¹ | SFS (mm) | PAF (%) | IMF (%) | IFB (mm) |
|------------------------|---------------------|--------------------|--------------------|----------------------|
| CTR | 4.52 ^a | 2.18 ^a | 3.67 ^d | 14.70 ^c |
| NA20 | 4.46 ^{ab} | 2.10 ^a | 3.70 ^d | 14.83 ^{bc} |
| NA40 | 4.39 ^{abc} | 1.88 ^b | 3.75 ^c | 15.12 ^{abc} |
| NA60 | 4.35 ^{bc} | 1.82 ^{bc} | 3.92 ^a | 15.35 ^{abc} |
| NA80 | 4.34 ^{bc} | 1.74 ^{cd} | 3.91 ^{ab} | 15.93 ^a |
| NA100 | 4.30 ^c | 1.70 ^d | 3.86 ^b | 15.96 ^a |
| SEM | 0.020 | 0.032 | 0.018 | 0.134 |
| <i>P</i> -value | | | | |
| ANOVA | 0.008 | <0.001 | <0.001 | 0.009 |
| Linear | <0.001 | <0.001 | <0.001 | <0.001 |
| Quadratic | 0.361 | 0.105 | 0.001 | 0.797 |

Abbreviations: IFB, intermuscular fat band; IMF, intramuscular fat; PAF, percentage of abdominal fat; SFS, subcutaneous fat plus skin.

^{a-d}Means within the same column with different superscripts differ significantly ($P < 0.05$).

¹CTR: basal diet; NA20, NA40, NA60, NA80, and NA100 group, basal diet adding 20, 40, 60, 80, and 100 mg/kg nicotinic acid, respectively.

supplementation of NA linearly increased water-holding capacity of breast muscle ($P < 0.001$), with the maxima measured in the NA80 and NA100 groups.

Carcass Characteristics

As shown in Table 4, the thickness of SFS was linearly decreased by the addition of NA in feed ($P < 0.001$), and the minimum occurred in the NA100 group. Increasing levels of dietary NA resulted in lower PAF in a linear manner ($P < 0.001$). Dietary NA supplementation significantly decreased PAF in all groups except NA20 in comparison to the CTR group ($P < 0.001$). There was a linear ($P < 0.001$) and quadratic ($P = 0.001$) increase in IMF content with the supplementation of NA, and the maximum occurred in the NA60 group. The width of IFB was linearly increased in response to the increase of NA supplementation ($P < 0.001$), and NA80 and NA100 groups had the highest values.

Lipid Metabolism

As shown in Table 5, serum TCH level was linearly decreased with the supplementation of NA ($P = 0.001$),

Table 3. Effects of nicotinic acid on meat quality of Wulong geese ($n = 6$).

| Treatment ¹ | Flesh color | | | pH | Shear force (kg/cm ²) | Drip loss (%) | Water-holding capacity (%) |
|------------------------|----------------------|-------|-------|--------------------|-----------------------------------|---------------|----------------------------|
| | L^* | a^* | b^* | | | | |
| CTR | 41.81 ^a | 14.36 | 3.94 | 6.11 ^b | 3.99 ^a | 3.70 | 69.87 ^c |
| NA20 | 41.29 ^{ab} | 14.39 | 3.86 | 6.21 ^{ab} | 3.95 ^a | 3.66 | 69.86 ^c |
| NA40 | 40.86 ^{abc} | 14.58 | 4.10 | 6.22 ^{ab} | 3.78 ^b | 3.64 | 70.09 ^{ab} |
| NA60 | 40.55 ^{bc} | 15.13 | 3.44 | 6.29 ^{ab} | 3.66 ^c | 3.57 | 70.11 ^{ab} |
| NA80 | 39.72 ^c | 14.50 | 3.53 | 6.33 ^a | 3.64 ^c | 3.55 | 70.16 ^a |
| NA100 | 39.64 ^c | 14.53 | 3.73 | 6.26 ^{ab} | 3.72 ^{bc} | 3.58 | 70.14 ^a |
| SEM | 0.208 | 0.116 | 0.098 | 0.022 | 0.026 | 0.024 | 0.032 |
| <i>P</i> -value | | | | | | | |
| ANOVA | 0.006 | 0.457 | 0.372 | 0.044 | <0.001 | 0.485 | 0.004 |
| Linear | <0.001 | 0.479 | 0.185 | 0.006 | <0.001 | 0.064 | <0.001 |
| Quadratic | 0.888 | 0.224 | 0.699 | 0.094 | <0.001 | 0.536 | 0.196 |

Abbreviations: L^* , lightness; a^* , redness; b^* , yellowness.

^{a-c}Means within the same column with different superscripts differ significantly ($P < 0.05$).

¹CTR: basal diet; NA20, NA40, NA60, NA80 and NA100 group, basal diet adding 20, 40, 60, 80 and 100 mg/kg nicotinic acid, respectively.

Table 5. Effects of nicotinic acid on serum lipid metabolism and liver lipoprotein lipase mRNA expression of Wulong geese (n = 6).

| Treatment ¹ | TCH (mmol/L) | TG (mmol/L) | LDL-C (mmol/L) | HDL-C (mmol/L) | MDH (U/mL) | Lipase (U/L) | LPL mRNA |
|------------------------|---------------------|--------------------|--------------------|-------------------|-------------------|----------------------|--------------------|
| CTR | 4.52 ^a | 1.10 ^a | 2.22 ^a | 1.67 ^c | 0.72 ^a | 68.88 ^d | 1.00 ^d |
| NA20 | 4.13 ^{abc} | 1.06 ^b | 2.01 ^{ab} | 1.70 ^c | 0.69 ^a | 71.53 ^{cd} | 1.11 ^{cd} |
| NA40 | 3.98 ^{bc} | 1.03 ^b | 1.94 ^{ab} | 1.75 ^b | 0.62 ^b | 73.87 ^{bcd} | 1.21 ^{bc} |
| NA60 | 3.78 ^c | 0.94 ^d | 1.90 ^{ab} | 1.85 ^a | 0.56 ^c | 82.16 ^a | 1.37 ^{ab} |
| NA80 | 3.71 ^c | 0.95 ^{cd} | 1.77 ^{bc} | 1.86 ^a | 0.54 ^c | 79.41 ^{ab} | 1.47 ^a |
| NA100 | 3.74 ^c | 0.98 ^c | 1.32 ^c | 1.82 ^a | 0.55 ^c | 77.67 ^{abc} | 1.42 ^{ab} |
| SEM | 0.080 | 0.011 | 0.084 | 0.014 | 0.014 | 1.201 | 0.041 |
| <i>P</i> -value | | | | | | | |
| ANOVA | 0.015 | <0.001 | 0.039 | <0.001 | <0.001 | 0.005 | <0.001 |
| Linear | 0.001 | <0.001 | 0.002 | <0.001 | <0.001 | 0.001 | <0.001 |
| Quadratic | 0.133 | <0.001 | 0.403 | 0.001 | 0.041 | 0.067 | 0.635 |

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LPL, lipoprotein lipase; MDH, malate dehydrogenase; TCH, total cholesterol; TG, triglyceride.

^{a-d}Means within the same column with different superscripts differ significantly ($P < 0.05$).

¹CTR: basal diet; NA20, NA40, NA60, NA80 and NA100 group, basal diet adding 20, 40, 60, 80 and 100 mg/kg nicotinic acid, respectively.

and the minima occurred in the NA60, NA80, and NA100 groups. Increasing levels of dietary NA decreased serum TG level in a linear ($P < 0.001$) and quadratic ($P < 0.001$) manner, with the minimum measured in the NA60 group. Serum LDL-C level was linearly decreased by the addition of NA ($P = 0.002$), and the NA100 group had the minimum. There was a linear ($P < 0.001$) and quadratic ($P = 0.001$) increase in the serum HDL-C level with the supplementation of NA, and the maxima were obtained in the NA60, NA80, and NA100 groups ($P > 0.05$). Dietary NA levels decreased the serum MDH activity in a linear ($P < 0.001$) and quadratic ($P = 0.041$) manner, with the minima occurred in NA60, NA80, and NA100 groups. Serum lipase activity was increased by the increased levels of dietary NA (linear, $P = 0.001$; quadratic, $P = 0.067$), and the highest lipase activity was observed in the NA60 group. Geese fed with NA supplemented-diets linearly increased the liver LPL mRNA expression ($P < 0.001$), and the maximum occurred in the NA80 group.

Tibia Parameters

As presented in Table 6, the tibia length was linearly increased as the dietary NA levels increased ($P < 0.001$), and the maxima were obtained in the NA80 and NA100

groups. Dietary NA supplementation linearly increased tibia circumference ($P < 0.001$), and the NA80 group had the highest value. The fat-free dry weight of tibia was increased by dietary NA supplementation in a linear manner ($P < 0.001$), and the maxima occurred in the NA60, NA80, and NA100 groups. There was a linear ($P < 0.001$) and quadratic ($P = 0.036$) increase in tibial Ca content with the increasing levels of NA addition. The maximal effects were obtained in the NA60 group. Tibial P content was increased with dietary NA supplementation in a linear ($P < 0.001$) and quadratic ($P < 0.001$) manner. All experimental groups except NA20 increased tibial P content in comparison with the CTR group ($P < 0.001$).

Quadratic Regression Analysis

As shown in Table 7, the data of shear force, IMF content, serum TG and HDL-C levels, and tibial Ca and P contents were selected for further analysis by quadratic regressions related to the dietary NA levels. The optimal NA level that minimized shear force of breast meat, and serum TG and MDH levels of geese was 79.69, 87.57, and 107.64 mg/kg, respectively. A maximum response for IMF content, serum HDL-C level, and tibial Ca and P contents was observed at 86.60, 88.14, 87.50, and 85.00 mg/kg, respectively.

Table 6. Effects of nicotinic acid on tibia parameters of Wulong geese (n = 6).

| Treatment ¹ | Length (cm) | Circumference (cm) | Fat-free dry weight (g) | Ash content (%) | Ca content (%) | P content (%) |
|------------------------|----------------------|--------------------|-------------------------|---------------------|---------------------|--------------------|
| CTR | 14.35 ^c | 3.53 ^c | 9.46 ^c | 57.66 ^c | 20.86 ^c | 10.36 ^c |
| NA20 | 14.72 ^{bc} | 3.56 ^c | 9.51 ^c | 57.46 ^c | 20.38 ^c | 10.86 ^b |
| NA40 | 14.96 ^{abc} | 3.63 ^{bc} | 9.88 ^{ab} | 57.82 ^c | 21.07 ^b | 11.25 ^a |
| NA60 | 15.16 ^{ab} | 3.64 ^{bc} | 10.05 ^a | 58.48 ^{bc} | 21.82 ^a | 11.52 ^a |
| NA80 | 15.50 ^a | 3.86 ^a | 10.13 ^a | 60.44 ^a | 21.36 ^{ab} | 11.61 ^a |
| NA100 | 15.42 ^a | 3.78 ^{ab} | 10.10 ^a | 60.18 ^a | 21.44 ^{ab} | 11.32 ^a |
| SEM | 0.102 | 0.027 | 0.061 | 0.291 | 0.131 | 0.085 |
| <i>P</i> -value | | | | | | |
| ANOVA | 0.003 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Linear | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Quadratic | 0.328 | 0.901 | 0.126 | 0.233 | 0.036 | <0.001 |

^{a-c}Means within the same row with different superscripts differ significantly ($P < 0.05$).

¹CTR: basal diet; NA20, NA40, NA60, NA80, and NA100 group, basal diet adding 20, 40, 60, 80, and 100 mg/kg nicotinic acid, respectively.

Table 7. Estimations of the extremum response for dietary nicotinic acid levels based on quadratic regressions in Wulong geese.

| Dependent variables | Regression equation ¹ | R ² | P | Extremum nicotinic acid response (mg/kg) ² |
|---------------------|--|----------------|--------|---|
| Shear force | $Y = 5.647 \times 10^{-5}X^2 - 0.009X + 4.037$ | 0.721 | <0.001 | 79.69 |
| IMF | $Y = -2.887 \times 10^{-5}X^2 + 0.005X + 3.637$ | 0.701 | 0.001 | 86.60 |
| TG | $Y = 2.284 \times 10^{-5}X^2 - 0.004X + 1.112$ | 0.713 | <0.001 | 87.57 |
| HDL-C | $Y = -2.269 \times 10^{-5}X^2 + 0.004X + 1.646$ | 0.716 | 0.001 | 88.14 |
| MDH | $Y = 1.858 \times 10^{-5}X^2 - 0.004X + 0.734$ | 0.648 | 0.041 | 107.64 |
| Ca | $Y = -2.000 \times 10^{-4}X^2 + 0.035X + 20.087$ | 0.441 | 0.036 | 87.50 |
| P | $Y = -2.000 \times 10^{-4}X^2 + 0.034X + 10.319$ | 0.713 | <0.001 | 85.00 |

Abbreviations: HDL-C, high-density lipoprotein cholesterol; IMF, intramuscular fat; MDH, malate dehydrogenase; TG, triglyceride.

¹Y is the dependent viable and X is the dietary nicotinic acid levels (mg/kg).

²Extremum nicotinic acid response is the maximum or minimum response of dietary nicotinic acid levels according to each regression equation (mg/kg).

DISCUSSION

Meat color is an important commercial characterization affecting consumers' purchasing decision. In this study, no significant effects were observed on a* and b* values in breast muscles, but NA supplementation linearly decreased L* value, and the minima were observed in the geese fed diets supplemented with 80 and 100 mg/kg NA. The L* value is used as an indicator of lightness degree, and a higher L* value indicates lighter meat (Tong et al., 2015). Our observations were in accordance with the report of Jiang et al. (2011), in which NA supplementation decreased the L* values of breast muscles in chicken. The change of meat pH value is used to evaluate the degree of glycogenolysis by glycosome after slaughter (Yang et al., 2016). The current study displayed that NA addition linearly increased the meat pH value, and diet supplemented with 80 mg/kg NA significantly increased the meat pH value compared with the basal diet. Similarly, Real et al. (2002) found that pigs fed NA had improved 24-h pH in longissimus. A delay in pH decline can lead to a reduction in protein denaturation, which can increase the water-holding capacity of breast muscle (Li et al., 2018). This explained the increased water-holding capacity in breast muscle from the NA-fed geese in the present study. In addition, meat pH value has been reported to be negatively correlated with L* value (Wyrwicz et al., 2012; Ijaz et al., 2020). Similarly, the decreased L* value and increased meat pH was observed with NA supplementation, especially in the diet supplemented with 80 mg/kg NA in our study.

Water-holding capacity is the ability of meat to retain its water during processing, storage, and cooking. The binding of protein to water limits the free space between swelling muscle fibers, and subsequently reduces the light reflectivity of muscle, as reflected by L* value (Wyrwicz et al., 2012). The similar conditions were also observed in our results, in which NA supplementation, especially at levels of 80 and 100 mg/kg, increased water-holding capacity and decreased L* value of breast muscle. Shear force is an important indicator to evaluate the tenderness of meat (Metheny et al., 2019). In this study, NA supplementation linearly and quadratically decreased the shear force. This was inconsistent with the results reported by Jiang et al. (2011), in which there was no significant effect of added NA on the shear force

of breast muscle in chicken. Previous studies have revealed that NA induced Type II (glycolytic) muscle fibers switching to Type I (oxidative) muscle fibers (Khan et al., 2013; Ringseis et al., 2013). Generally, muscles predominantly composed of Type I fibers tend to have lower glycolysis, higher ultimate pH and better tenderness (Kang et al., 2011). These results suggested that dietary NA supplementation was an effective way to improve meat quality of geese.

Abdominal and subcutaneous fat are the main sources of waste in the slaughterhouse, whereas fat stored intramuscularly is regarded as being a desirable trait related to meat quality in poultry (Liu et al., 2019; Yang et al., 2021). The success of poultry meat production has been strongly related to improvements in growth and carcass yield, mainly by increasing breast proportion and reducing abdominal fat (Zerehdaran et al., 2004). The content of IMF has become an important meat-quality trait and is positively related with meat flavor, juiciness, and tenderness (Zhang et al., 2021). Our data showed that NA supplementation, especially 80 and 100 mg/kg doses, decreased the thickness of SFS and PAF, and increased the content of IMF and width of IFB, which suggested an appropriate level of NA supplementation might retain IMF while decreasing the undesirable abdominal and subcutaneous fat. Yang et al. (2016) demonstrated that dietary supplementation of 1,000 mg/kg NA increased IMF content in steers, which might be due to the increased activities of glucose-6-phosphate dehydrogenase (G6PDH) and isocitrate dehydrogenase (ICDH), which facilitated gluconeogenesis and de novo fatty acid synthesis. In addition, Markovics and Tóth (2019) found that NA suppresses sebaceous lipogenesis of human sebocytes via activating hydroxycarboxylic acid receptor 2 (HCA2).

In this study, NA supplementation linearly reduced TCH, TG, and LDL-C levels and increased HDL-C level in the serum. Actually, NA has been used as a lipid-lowering drug in humans due to its effect on regulating serum lipids (Julius and Fischer, 2013; Romani et al., 2019). HDL plays a significant role in the process of reverse cholesterol transport and atheroprotective effects (Rader and Hovingh, 2014). However, a high-serum LDL level is associated with an increased risk of cardiovascular diseases (Ouyang et al., 2016). Similar to our results, in hypercholesterolemic diabetic and

nondiabetic rats, NA increased HDL-C but decreased TCH, TG, LDL-C, and total lipids (Zeb Shah et al., 2013). MDH is involved in the synthesis of NADPH, and NADPH is an important factor for de novo fatty acid synthesis (Lu et al., 2006). Previous studies demonstrated that hepatic MDH activity was positive correlated with abdominal fat weight in broilers (Chen et al., 2006; Chen et al., 2007). Similarly, our data showed that NA treatment decreased serum MDH activity as well as PAF. Lipase and LPL are essential for the utilization of lipid and facilitate TG lipolysis (He et al., 2013). In our study, NA treatment linearly increased serum lipase activity and hepatic *LPL* mRNA expression. These results suggested that the decreased SFS and PAF deposition might be due to the increased lipolysis and decreased fatty acid synthesis in geese.

Leg health is one of the significant issues related to the welfare of birds and profitability of production. Poultry with NA deficiency is susceptible to leg disease (Battig et al., 1953; Serafin, 1981). Battig et al. (1953) showed that a NA deficiency was responsible for perosis in goslings. This perotic condition was completely prevented by the addition of 40 mg/kg of NA. Tibia length, circumference, and weight are important bone developmental traits for poultry. Tibial ash, Ca, and P contents are reflective of the minerals, Ca, and P requirement necessary for growth and deposition in bone of poultry, respectively (Adeola and Walk, 2013). Our results displayed that NA supplementation increased the tibia parameters including length, circumference, fat-free dry weight and ash, Ca, and P contents. Previous studies noted that the potential beneficial mechanisms of action of niacin on bone were through its effects on *NAD-dependent deacetylase Sirtuin 1 (SIRT1)* gene expression and by diminishing inflammation (Iyer et al., 2014; Karacaglar et al., 2015). Conversely, Johnson et al. (1992) showed that feeding 1.5% NA decreased tibia strength and weights in chicks with no change in mineral content (Ca, P, Mg, Fe, and Zn) of the tibiae. Johnson et al. (1995) demonstrated that diets supplemented with 0.75 and 1% NA decreased tibia length and width of chicks compared with basal diet. These contradictory results might be caused by different dosages of NA and different kinds of experimental animals.

In conclusion, the present study showed that NA supplementation in the diet enhanced the meat quality, increased IMF content, regulated lipid metabolism, and increased tibia quality of Wulong geese. According to the quadratic regression analysis, a diet supplemented with 80 to 90 mg/kg NA would maximize the IMF content, serum HDL-C level, and tibial Ca and P contents, and minimize the muscle shear force and serum TG level. In view of economic costs, the dosage of 80 mg/kg NA in Wulong geese from 5 to 16 wk was recommended.

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DISCLOSURES

There are no conflicts of interest to declare.

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