Incidence rate of angel wing and its effect on wing bone development and serum biochemical parameters in geese

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ABSTRACT The first purpose of this study was to reveal the distribution of the angel wing (**AW**) of geese. Our data showed that the total incidence of AW was 6.67% in 150-day-old White Zhedong (**ZD**) geese, the occurrence of AW in left wing is higher than that in right wing and bilateral wing than unilateral wing (both $P < 0.01$). In 70-day-old Hybrid-Wanxi (**HW**) geese, the total incidence of AW was 8.86%, with similar incidence rate between unilateral and bilateral. The sex has not apparently affected the incidence of AW in both ZD and HW geese. To explore the potential relationship between wing type with body weight, organ index, bone characteristic, or blood biochemical parameters in 70-day-old HW geese. We found that the body weight and organ index were similar between normal wing (**NW**) and AW geese. The length for the humerus, metacarpal and phalanx, and the phalanx weights, as well as the angle between the humerus and the radial ulna (**HRU**) in NW geese were pronounced greater than that in AW geese ($P < 0.05$). Furthermore, the angel wing was strongly associated with lower platelet size indicators. Collectively, AW affected the wing bone length, phalanx weight, and HRU, and the occurrence of AW may be related with dysfunctional platelet activation in geese.

Key words: angel wing, bone characteristic, blood biochemistry, goose

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INTRODUCTION

Angel wing (**AW**) is characterized by outward twisting along the wrist joint on the unilateral or bilateral wing in birds, which is universal occurring in waterfowl including geese (Francis et al., 1967; Kreeger and Walser, 1984; Lin et al., 2016; Lin et al., 2017), swans (Mustafa et al., 2019), ducks (Shaw et al., 2012; Jeong et al., 2019), pelicans (Drew and Kreeger, 1986), and cormorants (Kuiken et al., 1999), even in other birds such as commercial chickens (Riddell, 1983), masked boobies (Pitman et al., 2012), Accipiter gentilis (Zsivanovits et al., 2006), and Grus americana (Vasseur et al., 2019). In addition to inferior appearance, AW also results in flight lessness in birds (Pitman et al., 2012; Vasseur et al., 2019) and compromises the birds' welfare (Rodenburg et al., 2005). Thus, it is important to understand the distribution of AW and its effect on wing bone development and serum biochemical parameters in geese.

The causes of AW may be varied. In waterfowl, it was considered that an excessive growth of the primary feathers placed greater pressure on the muscles and ligaments of the wrist joints and the metacarpal bone which is associated with malformation of the distal carpal bones and causing the forewings to twist outward (Kear and Janet, 1973; Anderson, 2004; Mustafa et al., 2019). Malnutrition may also play a role: high-protein diet, malnutrition, or insufficient calcium (**Ca**) and phosphorus (**P**) intake have previously been associated with AW (Serafin, 1982; Drew and Kreeger, 1986; Smith, 1997; Kuiken et al., 1999; Zsivanovits et al., 2006). The diet contained T-2 toxin and antioxidants increased the incidence and severity of AW in White Roman geese (Lin et al., 2017). The occurrence of AW is often linked to genetic. Francis et al. (1967) concluded that if inheritance involved in AW, the characteristic must be affected by more than one pair of genes. Lin et al. (2016) found that genetic selection of the AW phenotype aggravated the occurrence of AW in a study aimed to evaluate the effects of stocking density and genetic selection on the incidence of AW.

The aim of this study was to assess the distribution of AW which has occurred in White Zhedong (**ZD**) and Hybrid-Wanxi (**HW**) geese during its selection for rapid growth and to study the association of different wing

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types with body weight (**BW**), slaughter performance, wing bone development, and hematological parameters in HW geese.

MATERIALS AND METHODS

Animal Raising

The experimental protocol was approved by the Animal Care Committee of the Henan Agricultural University, China (18-0120). A total of 1-day-old 1,800 ZD geese and 800 HW geese (white Wanxi goose ♂ × white Taizhou goose ♀) were raised under the same conditions and given free access to food and drinking water. The goslings were raised in nursery during 1 to 28 d, followed by transferred to the growing house at the age of 28 d. The ambient temperature was maintained at approximately 33°C in the first week and then gradually lowered to 28°C based on normal management practices. The ZD geese (1−150 d) and HW geese (1−70 d) were fed the same based diet, which was formulated to meet or exceed nutrient requirements of goose according to National Research Council (1994), and was showed in Table 1. During the experimental period, normal immunization procedures were performed.

Distribution of Different Wing Types Geese

As illustrated with Figures 1A−1D, all AW geese were singled out from the population of HW geese at 70 d of age and ZD geese at 150 d of age, respectively. The distributions of different wing types, that is, unilateral angel wing (**UAW**) and bilateral angel wing (**BAW**), were recorded, and the AW ratios in different breed and sex were analyzed based on the population size. Some normal wing (**NW**) geese were randomly selected in corresponding population for comparing the difference of BW between AW (UAW and BAW) and selected NW geese.

Table 1. The nutritional level of the experimental diets (as-fed).

Nutrition composition	Experimental diet 0−4 wk	5−10 wk	11−22 wk
Ingredients, %			
Corn grain	58.96	66.03	66.23
Soybean meal	26.67	15.81	15.62
Bran	2.30	2.10	2.00
Alfalfa meal	7.10	11.05	11.10
Soybean oil	1.85	2.00	2.00
Limestone	0.65	0.50	0.70
$CaHPO_4$	1.06	1.10	1.15
L-lysine HCl	0.11	0.21	0.00
DL-methionine	0.10	0.00	0.00
NaCl	0.20	0.20	0.20
Premix [1]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Nutritional level, %			
Metabolic energy, Mcal/kg	2.90	3.00	3.00
Crude protein	19.00	15.00	15.00
Calcium	0.65	0.60	0.60
Digestibility phosphorus	0.30	0.30	0.30
Lysine	1.00	0.85	0.65
Methionine + cysteine	0.60	0.50	0.50

[1]Provided per kilogram of diet: Cu ($CuSO_4$•$5H_2O$), 8 mg; Fe ($FeSO_4$•$7H_2O$), 80 mg; Zn ($ZnSO_4$•$7H_2O$), 90 mg; Mn ($MnSO_4$•H_2O), 70 mg; Se ($NaSeO_3$), 0.3 mg; I (KI), 0.4 mg; Vitamin A, 9000 IU; Vitamin D_3, 1,600 IU; Vitamin E, 20 mg; Vitamin K_3, 2 mg; Vitamin B_{11}, 1.5 mg; Vitamin B_2, 4 mg; Vitamin B_6, 2 mg; niacin, 15 mg; folic acid, 0.6 mg; D-pantothenic acid, 10 mg; Vitamin B_{12}, 0.02 mg; biotin, 0.13 mg; choline, 1,000 mg.

Organs Indexes and the Angle of Wing of HW Geese

Ten males with different wing types (NW, UAW, and BAW) were randomly selected from the HW geese and slaughtered at 70 d of age, fasted for 12 h. The following index were measured, including BW, eviscerated weight, wing weight (of each side), and the weight of visceral organs. The angle of the carpal metacarpal and ulnar radius (**CMRU**) and the angle of the humerus and radial ulna (**HRU**) were measured with a sternogoiometer in accordance with the diagram in Figure 1E.

Morphological Analysis of Wing Bone in HW Goose

The right-wing bones from HW geese with NW (n = 8 −9) or BAW (n = 8−9) were removed for morphological characteristics analysis. The length and weight of wing bone were measured. Representative wings of different types from the same side in NW or BAW geese were scanned and photographed using an X-ray scanner (Kubtec, Stratford, CT).

Mechanical Test of Right-Wing Bone in HW Goose

The right-wing bones from HW geese with NW (n = 4) or AW (n = 4) were randomly selected for measurement of bone mineral content (**BMC**), bone mineral density (**BMD**), and mechanical strength. After wiping away the moisture and grease from the bones, BMC and BMD at different bone sites (humerus, ulna, radius, metacarpal, and phalanges) was analyzed with dual-energy X-ray absorptiometry (MEDIKORS, South Korea) with InAlyzer procedure according to the manufacturer's instructions. Then these bones were subjected to three-point bending test using texture analyzer (LP10K PLUS, Lloyd Instruments Ltd., UK). The load-deformation curve and the corresponding mechanical indexes were recorded including stiffness, ultimate load, ultimate strength and elastic modulus.

Ash, Ca, and P Content in Bones of HW Goose

Right-wing bones from NW (n = 4) and BAW (n = 4) phenotype of HW geese were degreased in ethyl ether and anhydrous ethanol for 24 h and dried at 100°C for 24 h. Subsequently, dry-defatted bone was ashed in a muffle furnace at 550°C for 24 h and the ash was measured on the basis of the percentage of dry-defatted

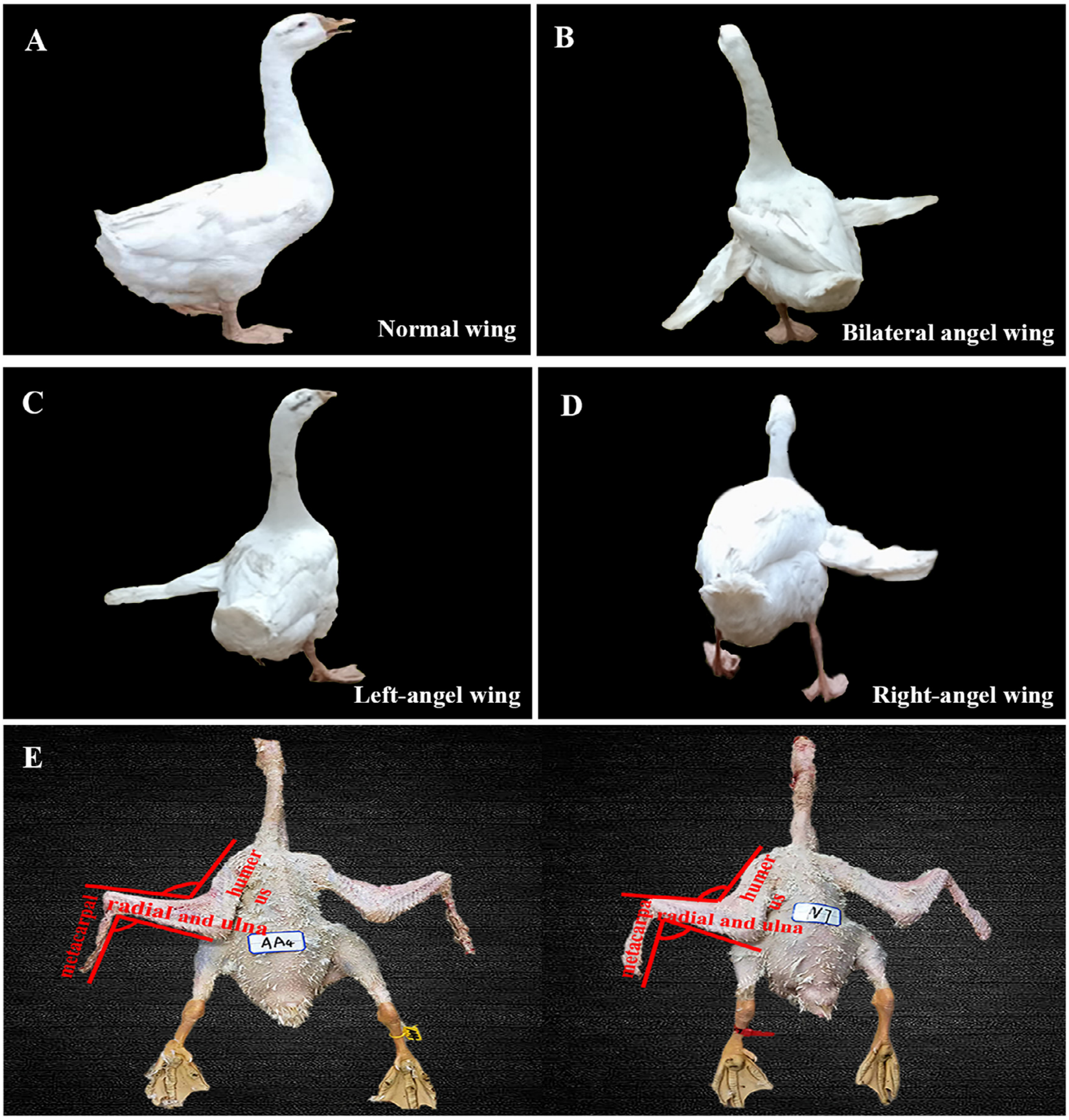

Figure 1. The represented geese with different wing types and location of measurements. (A) Normal wing, (B) bilateral angel wing, (C) left-angel wing, and (D) right-angel wing; (E) location of angle of wing.

weight (Guo et al., 2019). The ash was digested with hydrochloric acid and nitric acid, and P was determined with vanadium-molybdenum-yellow colorimetry. Ca was quantified with ethylene diamine tetraacetic acid complexometric titration (Sobczak et al., 2009).

Hematological Parameters of HW Geese

The whole blood (K_2EDTA-tube) was collected from the wing vein of NW, UAW, and BAW phenotype of HW geese ($n = 9-10$) for routine hematological parameter using the automatic hematology analyzer (Sysmex, Japan), including white blood cells (**WBC**), red blood cells (**RBC**), hemoglobin (**HGB**), neutrophils (**NEUT**), lymphocyte (**LYMPH**), monocytes (**MONO**), eosinophilic cell (**EO**), basophilic cell (**BASO**), hematocrit (**HCT**), mean corpuscular volume (**MCV**), mean corpuscular hemoglobin (**MHC**), mean corpuscular hemoglobin concentration (**MCHC**), platelet (**PLT**), thrombocytocrit (**PCT**), mean platelet volume (**MPV**), platelet ratio (**P-LCR**), and platelet distribution width (**PDW**). Serum was isolated from non-anticoagulant blood via centrifuged at 3,000 rpm/min for 10 min at 4°C for serum biochemical indicators determination. Serum total cholesterol (**TC**), high density lipoprotein (**HDL**), low density lipoprotein (**LDL**), triglycerides (**TG**), total protein (**TP**), albumin (**ALB**), globulin (**GLB**), Ca, P, creatine kinase (**CK**), alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), and alkaline phosphatase (**ALP**) were analyzed using Fully Automatic Biochemical Analyzer (Hitachi, Japan).

Statistical Analysis

All statistical analyses were performed in SPSS software (version 25.0). The data on the incidence of AW in different populations were subjected to chi-square analysis separately, the lengths of the wing bones were

Table 2. The incidence of AW in the two goose populations.

Population	Gender	UAW			BAW	Total
		LAW	RAW	Total		
ZD goose (n = 1,500)	Male	10 (0.67%)	5 (0.33%)	15 (1%)	40 (2.66%)	55 (3.66%)
	Female	16 (1.07%)	3 (0.2%)	19 (1.27%)	26 (1.74%)	45 (3.01%)
	Total	26 (1.74%)*	8 (0.53%)	34 (2.27%)	66 (4.4%)#	100 (6.67%)
HW goose (n = 700)	Male	6 (0.86%)	6 (0.86%)	12 (1.72%)	15 (2.15%)	27 (3.87%)
	Female	12 (1.71%)	11 (1.57%)	23 (3.28%)	12 (1.71%)	35 (4.99%)
	Total	18 (2.57%)	17 (2.43%)	35 (5%)	27 (3.86%)	62 (8.86%)

Abbreviations: BAW, goose with bilateral angel wing; HW, Hybrid-Wanxi at 70 d of age; LAW, goose with left-angel wing; RAW, goose with right-angel wing; UAW, goose with unilateral angel wing; ZD, White Zhedong at 150 d of age.
*Represent significant difference between LAW and RAW.
#Represent significant difference between UAW and BAW.

analyzed with one-way ANOVA with the body weight as the covariate, and the other data were analyzed with one-way ANOVA. Data were expressed as mean ± standard error of mean (**SEM**). Multiple comparisons were analyzed with the Bonferroni method. Differences were considered significant with P-values less than 0.05.

RESULTS

The Incidence of AW in Different Populations of Geese

As shown in Table 2, the total incidence of AW was 6.67 and 8.86% in ZD geese and HW geese, respectively. In the ZD geese, chi-square tests showed that the incidence of AW in bilateral wings was greater than that in unilateral wings ($\chi^2 = 10.24$, $P < 0.01$), and the occurrence rate of AW in left wings were remarkably higher than that in right wings in unilateral angel wing (**UAW**) geese ($\chi^2 = 9.53$, $P < 0.01$). However, there was no significant difference in the incidence of AW between male and female ($\chi^2 = 1.00$, $P = 0.32$). Regarding HW geese, the incidences of AW were similar between male and female ($\chi^2 = 1.03$, $P = 0.31$), and unilateral and bilateral ($\chi^2 = 0.03$, $P = 0.87$), as well as the left and right wings in UAW geese ($\chi^2 = 1.03$, $P = 0.31$).

BW Responses to Different Wing Type in Two Populations

The relationship between BW and different wing type of ZD and HW goose were illustrated in Table 3. The males weighted more than the females in both ZD and WH geese, whereas no significant differences were observed in BW among NW, UAW, and BAW geese regardless male or female ($P > 0.05$) in both ZD and HW geese population. Of note, a trend with NW > UAW > BAW in terms of BW was observed among the 3-wing type except for the female HW geese.

Organ Index Response to Wing Types in HW Goose

There was no notably difference in eviscerated weight, wing weight and percentage, and the absolute and relative weights of visceral organs among NW, UAW, and BAW geese ($P > 0.05$). However, we observed that the relative testis weight ($P = 0.092$) in BAW geese was somewhat larger than that in NW geese (Table 4).

Considering the distinct phenotypic difference among the 3 wing types of HW geese, we performed a separate comparison of the wing weight and percentage between NWs and AWs (Figure 2). In NW and BAW geese, the weight of NW was significantly greater than that of AW ($P < 0.05$, Figure 2A), but there was no apparent difference in the percentage of wing ($P > 0.05$, Figure 2B).

Angle of CMRU and HRU of Wing in HW Goose

To explore the difference of the angle in NW and AW, the date indicated that these birds possessed comparable the angle of CMRU (Figure 2C), whereas the angle of HRU in NW was significantly greater than that in AW ($P < 0.01$, Figure 2D)

The Weight and Length of Wing Bone Response to Wing Types in HW Goose

After normalizing the data to BW, we found that the phalanx weight of NW was significantly greater than

Table 3. Body weights among geese with different wing types in the two goose populations (kg).

Population	Gender	Wing type			SEM	P-value
		NW	UAW	BAW		
ZD goose	Male	5.08 (n = 10)	4.81 (n = 15)	4.75 (n = 40)	0.05	0.097
	Female	4.63 (n = 10)	4.55 (n = 19)	4.38 (n = 26)	0.07	0.385
HW goose	Male	4.34 (n = 21)	4.32 (n = 12)	4.18 (n = 17)	0.05	0.337
	Female	3.61 (n = 17)	3.60 (n = 23)	3.64 (n = 10)	0.05	0.972

Abbreviations: BAW, goose with bilateral angel wing; HW, Hybrid-Wanxi at 70 d of age; NW, goose with normal wing; UAW, goose with unilateral angel wing; ZD, White Zhedong at 150 d of age.

Table 4. The comparison of organs indexes among HW geese with different wing types.

Item	Weight	Wing type			SEM	P-value
		NW	UAW	BAW		
	Live weight (kg)	4.47	4.48	4.34	0.08	0.713
	Eviscerated weight(kg)	3.41	3.29	3.23	0.07	0.494
Wing	Total weight (kg)	0.48	0.43	0.44	0.01	0.148
	Percentage of wing (%)	13.98	13.20	13.55	0.18	0.217
Cardiac	Weight (g)	33.78	29.99	30.96	0.90	0.204
	Relative weight (g/kg)	7.57	6.70	7.12	0.17	0.116
Liver	Weight (g)	82.18	78.78	81.43	2.25	0.914
	Relative weight (g/kg)	18.45	17.85	18.74	0.46	0.746
Spleen	Weight (g)	4.63	3.93	4.79	0.55	0.441
	Relative weight (g/kg)	1.05	0.87	1.10	0.06	0.262
Testis	Weight (g)	0.29	0.30	0.40	0.03	0.132
	Relative weight (g/kg)	0.066	0.065	0.092	0.006	0.092

Abbreviations: BAW, goose with bilateral angel wing; NW, goose with normal wing; UAW, goose with unilateral angel wing.
Percentage of wing is the total weight of wings relative to eviscerated weight.
Relative weight of other traits is part weight relative to live weight. n = 7−10.

that of AW ($P = 0.012$). The lengths of humerus ($P = 0.037$), metacarpal ($P = 0.008$), and phalanx ($P = 0.008$) in NW were notably longer than those in AW (Table 5).

Morphological Change of Wing Bone of HW Goose

Autopsy and X-ray scanning showed no apparent differences in the humerus, ulna, radius, and phalanges bone between NW and AW (Figure 3A). However, a clear change was observed in the fourth metacarpal; the proximal metacarpal was normal, whereas the fourth metacarpal was rotated from the middle and continued steadily toward the distal end. The middle of the fourth metacarpal had a rotation of approximately 90°, as marked in a red box in Figure 3B.

Bone Quality Response to Wing Types in HW Goose

No obvious difference in BMC, BMD, stiffness, ultimate load, ultimate strength, and elastic modulus was observed in the humerus, ulna, radius, metacarpal, and

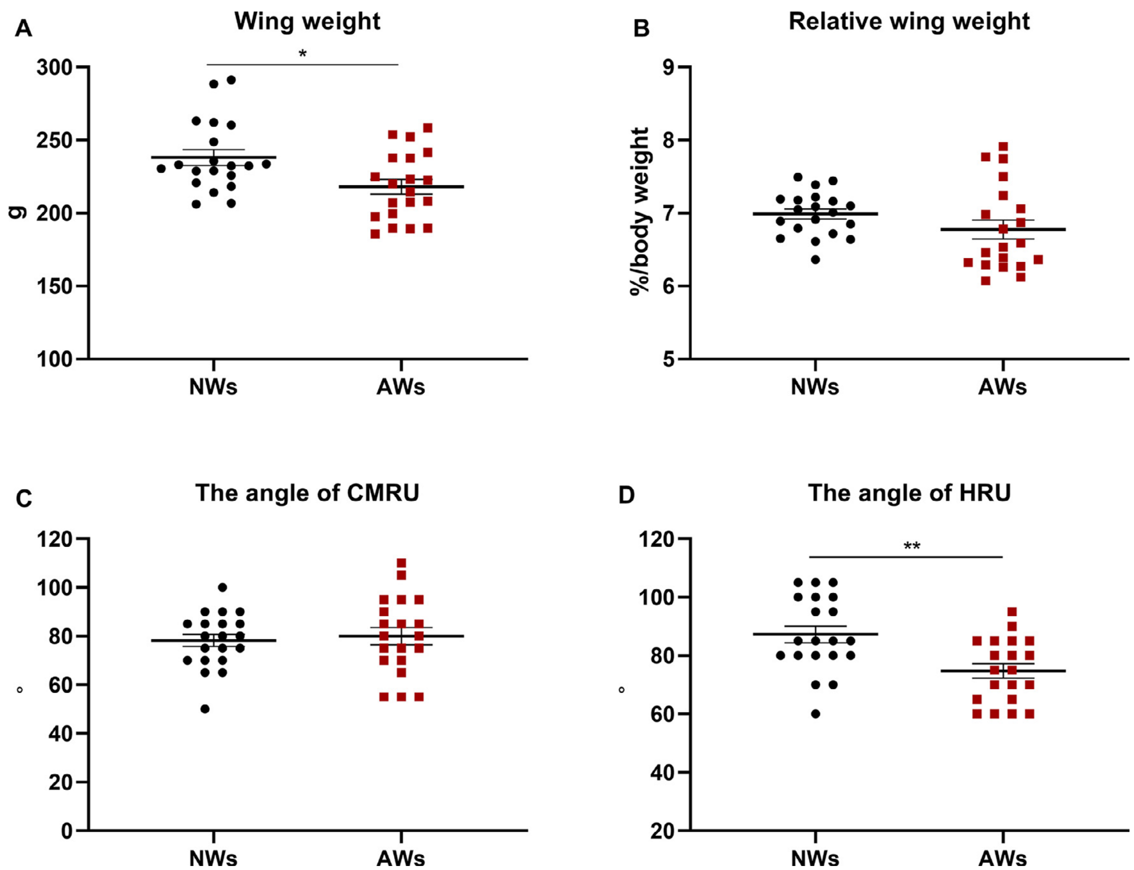

Figure 2. The weight and angle of wing for HW geese with different wing type. (A) Wing weight; (B) relative wing weight; (C) the angle between the carpal metacarpal the radial ulna (CMRU); (D) the angle between the humerus and the radial ulna (HRU). * $P < 0.05$, ** $P < 0.01$. The data from both-side wings were used. Abbreviations: AW, angel wings from bilateral angel wing geese; NW, normal wing from normal wing geese.

Table 5. The relative weights (g/kg) and length (mm) of the wing bone for NWs and AWs in HW geese.

Index	Parts	The kinds of wing NWs	AWs	SEM	*P*-value
Weight	Humerus	8.32	7.95	0.19	0.331
	Radioulnar	4.87	4.80	0.12	0.774
	Metacarpal	1.78	1.67	0.04	0.185
	Phalanges	0.46*	0.41	0.01	0.012
Length	Humerus	177.95*	173.51	0.90	0.037
	Ulna	161.90	158.88	1.07	0.205
	Radial	154.57	151.54	0.99	0.173
	Metacarpal	97.12**	93.35	0.57	0.008
	Phalanges	41.03**	39.11	0.29	0.008

NWs, normal wing form normal wing geese. AWs, angel wings from bilateral angel wing geese; wing were from the right-side wing of HW goose. n = 8−9.
*Means $P < 0.05$.
**Means $P < 0.01$.

Table 6. The bone mineral density in each wing bone in NW and AW in HW geese.

Parts	Parameter	The kinds of wing NWs	AWs	SEM	*P*-value
Humerus	BMC (g)	8.89	8.46	0.02	0.606
	BMD (g/cm^2)	0.25	0.26	0.01	0.495
Ulna	BMC (g)	3.47	3.50	0.02	0.922
	BMD (g/cm^2)	0.21	0.22	0.01	0.758
Radial	BMC (g)	1.75	1.80	0.07	0.740
	BMD (g/cm^2)	0.21	0.22	0.01	0.279
Metacarpal	BMC (g)	2.37	2.11	0.11	0.303
	BMD (g/cm^2)	0.22	0.22	0.01	0.680
Phalanges	BMC (g)	0.48	0.43	0.03	0.442
	BMD (g/cm^2)	0.11	0.11	0.00	0.764

Abbreviations: BMC, bone mineral content; BMD, bone mineral density.
NW, normal wings form normal wing geese; AW, angel wings from bilateral angel wing geese; wings were from the right-side wing of HW goose. n = 4

phalanx form NW and AW ($P > 0.05$, Tables 6 and 7). Also, there were no significant differences in the content of ash, Ca, and P between NW and AW ($P > 0.05$, Table 8).

Hematological Parameter Response to Wing Types in HW Goose

The date of routine blood parameters showed that the level of platelet size (MPV, P-LCR, and PDW) in NW geese were significantly higher than those in UAW and BAW geese, whereas there were no significant differences between UAW and BAW geese (Table 9). Moreover, no marketable differences in white blood cells parameters (WBC, NEUT, LYMPH, MONO, EO, and BASO), red blood cells parameters (RBC, HCT, and MCV), hemoglobin parameters (HGB, MCH, and MCHC) and other part of platelet parameters (PLT and PCT) were observed among NW, UAW, and BAW geese (Table 9). In addition, we also noticed that where no significant differences in serum lipid biochemical indicators (TC, HDL, LDL, and TG), serum protein biochemical indicators (TP, ALB, and GLB), inorganic ion biochemical indices (Ca and P) and enzyme indices (CK, ALT, AST, and ALP) among NW, UAW, and BAW geese (Table 10).

DISCUSSION

AW affects the appearance of geese (Pitman et al., 2012; Vasseur et al., 2019) and compromises the birds' welfare (Rodenburg et al., 2005). Estimates of the prevalence of AW in geese have been reported between 5 and 33% (Kuiken et al., 1999; Fan et al., 2006; Lin et al.,

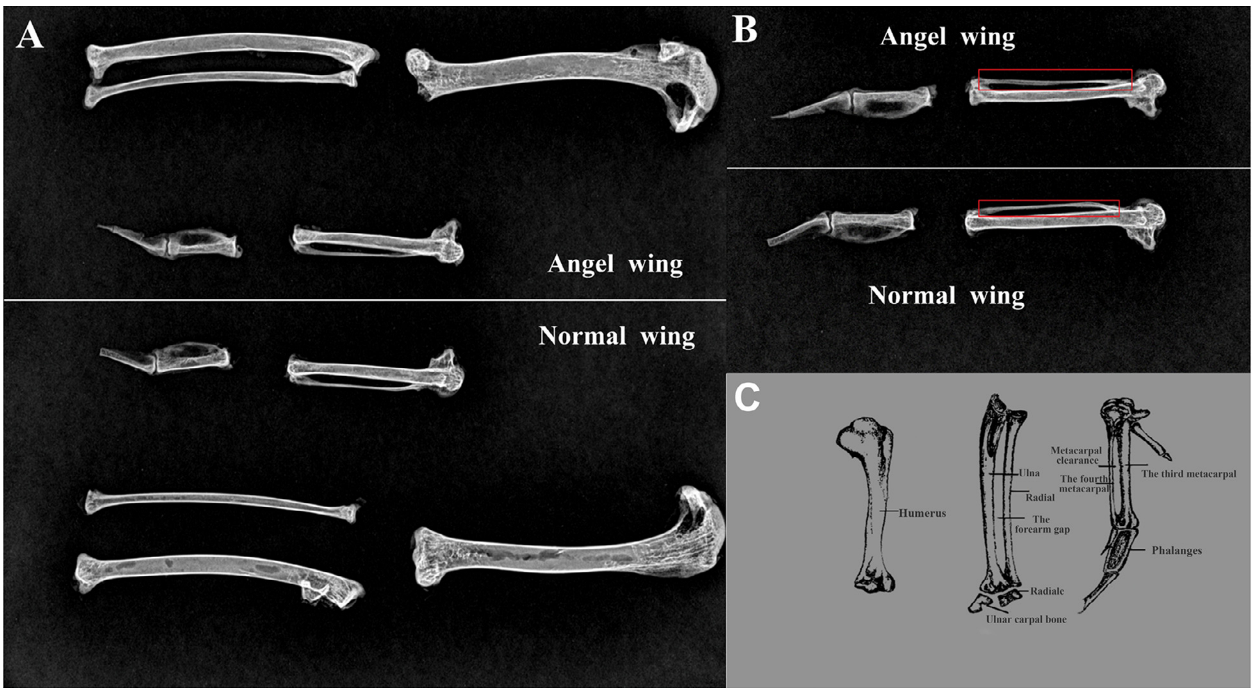

Figure 3. The results of X-ray scan of various parts of the wing. (A) The overall results of X-ray scan of humerus, ulna, radius, metacarpal and phalanx. (B) The X-ray scan of metacarpal and phalanges of the wing. (C) The skeleton names of each parts of the wing.

Table 7. The mechanical strength of each wing bone for NW and AW in HW geese.

Parts	Parameter	The kinds of wing NWs	AWs	SEM	*P*-value
Humerus	Stiffness (N/m)	72,243.43	127,757.22	15,984.43	0.077
	Ultimate load (N)	246.24	275.96	20.16	0.504
	Ultimate strength (MPa)	34.78	40.49	2.33	0.249
	Elastic modulus (MPa)	1,544.35	2,444.41	321.86	0.178
Ulna	Stiffness (N/m)	29,930.80	33,299.30	4,321.61	0.728
	Ultimate load (N)	71.09	61.49	0.04	0.611
	Ultimate strength (MPa)	115.86	87.68	13.25	0.324
	Elastic modulus (MPa)	10,406.32	7,756.01	1,322.77	0.355
Radial	Stiffness (N/m)	87,317.48	10,32,272.70	8,235.35	0.372
	Ultimate load (N)	176.47	160.20	8.15	0.357
	Ultimate strength (MPa)	119.71	83.25	11.72	0.125
	Elastic modulus (MPa)	7,562.61	6,261.47	918.42	0.521
Metacarpal	Stiffness (N/m)	129,620.65	113,692.01	11,238.08	0.521
	Ultimate load (N)	196.97	167.18	19.65	0.491
	Ultimate strength (MPa)	52.51	40.88	5.06	0.282
	Elastic modulus (MPa)	3,240.17	2,522.57	284.31	0.232
Phalanges	Stiffness (N/m)	124,235.66	149,002.29	23,822.94	0.641
	Ultimate load (N)	156.58	151.65	15.53	0.888
	Ultimate strength (MPa)	127.59	123.57	12.66	0.888
	Elastic modulus (MPa)	5,399.27	6,475.63	1,035.34	0.641

NW, normal wings form normal wing geese; AW, angel wings from bilateral angel wing geese; wings were from the right-side wing of HW goose. n = 4.

2008; Vasseur et al., 2019), even it might reach 71% in AW offspring (Lin et al., 2016). Various studies have reported figures for the prevalence of AW in offspring of goose. For instance, Lin et al. (2016) noticed an incidence of AW of 33.1% in the offspring of NW geese, and 71% in the offspring of AW geese. Francis et al. (1967) found an incidence of AW of 14.7 and 53% in the offspring of NW and AW geese, respectively. In the present study, the incidence of AW was 6.67% in ZD geese and 8.86% in HW geese, indicating that AW occurs in all types of goose populations. Based on published data, the variation in AW incidence may be related to genetic and/or nutritional factors. Furthermore, our results showed that AW was more likely to occur bilaterally than unilaterally, the left wing has higher incidence than right wing as far as UAW is concerned in 150-day-old ZD geese, whereas there was similar incidence rate between unilateral and bilateral in 70-day-old HW geese, which might be explained by the breed heterogeneity or AW phenotype did not appear fully in 70 day-old HW geese. In line with these results, Pitman et al. (2012) reported that AW occurs unilaterally or bilaterally in masked boobies, and the left wing has been reported to be more commonly affected than the right wing (Kear and Janet, 1973; Kreeger and Walser, 1984). In addition, on the basis of sex identification through anal examination in 2 separate commercial populations, the incidence of AW was similar between

Table 8. The content (%) of ash, Ca, and P in each wing bone in NWs and AWs in HW geese.

Tissue	Item	The kinds of wing NWs	AWs	SEM	*P*-value
Humerus	Crude ash	36.38	38.34	0.71	0.183
	Ca	13.58	14.30	0.90	0.720
	P	5.59	5.88	0.13	0.298
Ulna	Crude ash	39.59	41.76	1.14	0.380
	Ca	12.91	14.43	0.58	0.208
	P	5.95	6.20	0.15	0.445
Radial	Crude ash	41.92	43.95	0.84	0.256
	Ca	15.60	14.94	0.56	0.597
	P	5.81	6.08	0.15	0.422
Metacarpal	Crude ash	39.83	42.39	1.04	0.250
	Ca	15.71	16.24	0.68	0.726
	P	5.95	5.78	0.15	0.634
Phalanges	Crude ash	37.52	37.88	1.05	0.945
	Ca	12.27	13.03	0.57	0.549
	P	5.95	6.30	0.26	0.546

Abbreviations: Ca, calcium, P, phosphorus.

NW, normal wings form normal wing geese; AW, angel wings from bilateral angel wing geese; wings were from the right-side wing of HW goose. n = 4.

Table 9. The routine blood parameters among HW geese with different wing types.

Item	Population NW	UAW	BAW	SEM	*P*-value
WBC (10^9/L)	36.16	42.34	40.37	1.58	0.286
NEUT (10^9/L)	1.84	3.08	1.28	0.40	0.185
LYMPH (10^9/L)	1.94	1.88	2.57	0.48	0.807
MONO (10^9/L)	0.06	0.11	0.07	0.02	0.598
E0 (10^9/L)	0.11	0.08	0.15	0.02	0.470
BASO (10^7/L)	0.22	0.11	0.70	0.14	0.174
RBC (10^{12}/L)	1.36	1.47	1.40	0.06	0.797
HGB (g/L)	97.78	110.67	108.60	2.94	0.167
HCT (%)	21.33	22.88	22.30	0.92	0.804
MCV (fL)	157.02	155.49	161.41	2.32	0.566
MCH (pg)	76.72	76.52	83.50	4.34	0.763
MCHC (g/L)	487.44	492.00	513.70	25.09	0.905
PLT (10^9/L)	69.44	78.89	72.50	9.10	0.919
MPV (fL)	8.80^a	7.87^b	7.81^b	0.14	0.02
PCT (%)	0.063	0.062	0.057	0.01	0.935
P-LCR (%)	17.46^a	11.36^b	10.79^b	0.87	0.001
PDW (fL)	8.24^a	6.97^b	6.89^b	0.17	0.00

Abbreviations: BASO, basophilic cell; BAW, goose with bilateral angel wing; EO, eosinophilic cell; HGB, hemoglobin; HCT, hematocrit; LYMPH, lymphocyte; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; MONO, monocytes; NEUT, neutrophils; NW, goose with normal wing; PLT, platelet; PCT, thrombocytocrit; P-LCR, platelet ratio; PDW, platelet distribution width; RBC, red blood cells; UAW, goose with unilateral angel wing; WBC, white blood cells.

[a,b]Values with different small letter superscripts mean $P < 0.05$. n = 9−10.

Table 10. The serum biochemical parameters among HW geese with different wing types,

Item	Population NW	UAW	BAW	SEM	*P*-value
CK (U/L)	848.10	1,082.00	783.50	67.54	0.178
ALT (U/L)	14.80	13.89	15.00	0.73	0.820
AST (U/L)	29.30	27.11	27.90	1.10	0.725
ALP (U/L)	498.50	700.78	567.90	48.35	0.238
TP (g/L)	47.60	47.81	48.99	1.26	0.894
ALB (g/L)	12.91	13.11	13.02	0.20	0.928
GLB (g/L)	34.69	34.70	35.97	1.11	0.870
TC (mmol/L)	5.55	5.66	5.81	0.13	0.724
HDL (mmol/L)	3.72	4.00	3.75	0.10	0.466
LDL (mmol/L)	1.71	1.61	1.96	0.07	0.136
Ca (mmol/L)	2.94	2.87	2.87	0.04	0.706
P (mmol/L)	2.52	2.36	2.16	0.13	0.542
TG (mmol/L)	0.70	0.68	0.70	0.04	0.960

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BAW, goose with bilateral angel wing; Ca, calcium; CK, creatine kinase; GLB, globulin; HDL, high density lipoprotein; LDL, low density lipoprotein; NW, goose with normal wing; P, phosphorus; TC, total cholesterol; TG, triglycerides; TP, total protein; UAW, goose with unilateral angel wing. n = 9-10.

males and females in both ZD and HW geese in this study.

The causes of AW are considered to be associated with an enhanced growth rate, that is, rapid weight gain might result in faster wing growth, and consequently caused the higher occurrence of AW in heavier than lighter animals (Kear and Janet, 1973; Serafin 1982; Kuiken et al., 1999), which is inconsistent with our study. In this regard, we observed no significant difference for BW with different wing types in both ZD and HW geese. Lin et al. (2016) also reported no differences in BW between the offspring of AW and NW geese before 12 wk of age, although the BW of AW geese appear to slightly decrease in 14 wk-old geese.

Considering the bone characteristics, a notably decrease in the length of humerus, metacarpal and phalanges, and the weight of phalanx, along with a slightly decline in the wing weight suggested the effect of AW on wing bone. These data also indicated that the decrease in the weight and length of wing bones might contribute to reducing the body overloads. As reported by Kreeger and Walser (1984), the results of autopsy and X-ray scanning in the present study also confirmed the main abnormality in the fourth metacarpal bone in AW. Wing bone deformation may alter BMC, strength or density of bone. However, we did not find difference between NW and AW in bone related indicators including BMD, and bone mechanical force, the content of Ca and P in wing bone. In this context, we deemed that AW did not significantly affect the internal structure and intrinsic properties of each wing bone. Interestingly, we observed a trend in which the metacarpal stiffness in AW was slightly lower than NW. Conversely, the stiffness in the humerus, ulna, radius and phalanx in AW was weakly stronger than in NW. Combining with the date of X-ray scanning manifesting the metacarpal shape in AW was different from that in NW, we speculate that a change in the metacarpal shape in AW may decrease the metacarpal stiffness and result in a compensatory increase in strength of the humerus, ulna, radius, and phalanges to achieve a balance in wing bone to support the wing.

Abnormal wing development may give rise to alternation in hematological parameters, which are important indexes reflecting the health status of animals (Chen et al., 2019). Recent studies have found that the occurring of AW was accompanied by abnormal hematologic parameters (leukocyte, lymphocyte, granulocyte, and monocyte) in swan (Mustafa et al., 2019). In the current study, no apparent difference in leukocyte, lymphocyte, granulocyte, and monocyte was observed among NW, UAW, and BAW geese, whereas the platelet size (MPV, P-LCR, and PDW) in NW geese was significantly greater than those in AW geese. Platelets are produced in the bone marrow, platelet size (MPV, PDW, and P-LCR) was considered as markers of platelet activation and potential clinical biomarker reflecting platelet-related inflammation (Ulusoy et al., 2018; Cao et al., 2020). Platelets-activating factor stimulates proliferation of bone cells, activated platelets can release growth factors favoring bone formation and bone remodeling (Cenni et al., 2002; Gruber et al., 2002; Sharif and Abdollahi, 2010; Rodriguez et al., 2014). Therefore, decreased platelet size in AW geese may impair platelet activation and thus affected bone formation, which might in turn induce the occurrence of AW.

A limitation of the present study is that the experiment design, this research just paid more attention to the effect of AW on wing bone development and serum biochemical parameters in 70-day-old HW geese. During this period, we also noticed that ZD geese is more susceptive than HW geese in terms of the incidence of AW with considerable difference in BW between NW and AW (UAW or BAW) geese, which seem to be more meaningful to explore the side-effects of AW. Alternatively, a 2 × 2 factorial experiment with 2 breeds and 2 ages seems to be logic to investigate the effects of breed and age on the incidence rate of AW. Further experiments would be essential to illustrate this possibility.

From the abovementioned results, it could be concluded that the probability of incidence of AW differed from breeds but not sex, and the AW appears to be associated with decreased the lengths of humerus, metacarpal, and phalanges, as well as the phalanx weight. Furthermore, the occurrence of AW phenotype may be related with dysfunctional platelet activation in geese.

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DISCLOSURES

The authors declare that they have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service or company that could be construed as influencing the position presented in the manuscript entitled “Incidence rate of angel wing and its effect on wing bone development and serum biochemical parameters in geese”.

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