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ARTICLE

Effects of Animal Welfare-Certified Rearing Systems on the Blood Parameters and Meat Quality Characteristics of Broilers at the Farm Level in Korea

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Abstract Compared to the conventional farms (CF) rearing of broilers, the rearing management of animal welfare-certified farms (AF) must provide low stocking density, perch, air regulation, and feeding plant-based protein. This study aimed to compare the effects of rearing management in CF and AF on blood parameters, meat quality, and bioactive compound content in Ross 308 broiler chickens at the farm level before transportation to slaughterhouses. Blood and meat samples were obtained at 28–35-day-old chickens from three CF and three AF. In blood samples, low-density lipoprotein cholesterol ($p<0.05$), triglyceride ($p<0.001$), glucose ($p<0.01$), total protein ($p<0.001$), albumin ($p<0.01$), and white blood cell ($p<0.001$) levels as well as the heterophil/lymphocyte ratio [stress index (SI), $p<0.001$] were lower in broilers from AF than in CF. In meat samples, shear force ($p<0.001$, $p<0.05$), and carnosine contents ($p<0.001$, $p<0.05$) in both breast and thigh meat from AF were higher than those in meat from CF. The contents of linoleic acid ($p<0.001$), α-linolenic acid ($p<0.001$), and eicosapentaenoic acid ($p<0.05$) were higher in the samples from AF than those from CF. This study reveals that such differences are influenced by the different rearing factors in nutrition, housing, and management practices between CF and AF. Supplementation of plant-based protein and enough space to move due to lower stocking density accounts for the large differences between them. These results can be used as preliminary data showing that the AF system reduces the SI and enhances carnosine and polyunsaturated fatty acids levels in chicken meat at the farm level before transportation.

Keywords broiler, conventional, welfare, stress index, meat quality

Introduction

With consumers' growing interest in the origin of their food and the conditions in which the animals have been reared, there is an increasing demand for animal ethics and welfare among the consumers. Therefore, the animal welfare certification system for broilers was introduced in South Korea in 2014. Chicken products can be certified only when the animal welfare criteria for broilers by Animal and Plant Quarantine Agency (APQA, 2018) are met throughout the whole process, from farming to hatching, transportation, lairage, and slaughter phases. Unlike conventional broiler farms (CF), animal welfare-certified broiler farms (AF) need to be equipped with conditions such as low stocking density (less than 19 birds or less than 30 kg/m^2), 2 m length perch for 1,000 broilers, plant-based protein in feeds and environmental enrichments (straw, wood shaving bales, and vegetables), lighting program (minimum 8 h of continuous light and at least 6 h of continuous darkness), lighting intensity (minimum 20 Lx), and air quality (NH_3<20 ppm, CO_2<5,000 ppm). Such certification standards are implemented to improve the welfare of broilers.

Several studies have shown that increased activity, especially at an earlier age, can reinforce the bones and muscles of broilers (Bizeray et al., 2002a). Stocking density can affect the development of the broiler chickens (Buijs et al., 2012) and a higher density indicates lower activity leading to broiler leg health problems. Perches offer broilers the third spatial dimension that can increase exercise while jumping on and off as broilers move around (Bizeray et al., 2002b). Meluzzi and Sirri (2009) reported that behavioral activities were significantly increased when birds were provided with a chance to climb, scratch, and perch. Nevertheless, environmental enrichment, including perch, can improve the welfare of broilers by accommodating a larger space and enticing them to perform more of their species-specific behavior (van de Weerd and Day, 2009). As the legislation of protein source for chickens is set out as “mammalian or avian-derived protein-free” for diets (RSPCA, 2017) and a supply of animal protein is limited in some regions, resulting in the increasing trend of providing poultry diets of plant material or vegetable protein sources worldwide.

The chicken meat from AF, meeting these rearing conditions is about 20% more expensive than that from CF. Consumers who choose animal welfare-certified chicken products despite the high prices are expecting that chicken meat from AF should have superior safety, hygiene, and quality improvement to chicken meat from CF (Alonso et al., 2020). Song et al. (2014) reported that consumers who choose animal welfare livestock products more considered freshness, tasty and quality of meat than those who were not. From the results of a questionnaire in Korea answered by 1,000 people, 85.5% and 9.3% of people selected animal welfare chicken products expecting safety and freshness of meat, respectively (Lee, 2017). However, related research regarding the impacts of different rearing management methods in AF and CF on the welfare and meat quality of broilers at the farm level is still lacking. Previous studies mainly compared the meat quality of the broilers affected by transportation and lairage environment (Kim et al., 2020), however, no previous studies have assessed the blood profile and meat quality of broilers at the farm level before the loading, transportation, and lairage.

Silva et al. (2020) reported that values of the hematology and blood chemistry including H/L ratio in animals are key factors to assess their health and welfare status, and the study of Tagliari et al. (2010) showed a significant increase of 2-thiobarbituric acid reactive substances (TBARS) in stressed animals. Therefore, this study was conducted to assess only the effect of two different rearing systems on blood parameters, stress levels, and meat quality of broiler chickens at the farm level for the first time in Korea.

Materials and Methods

Birds, housing, and feeding

We selected three CFs and three AFs in the Jeolla-do Province in Korea. Farm information constituting size, housing type, enrichment, insulation facilities, slaughter age, and weight of broilers are shown in Table 1. Unlike conventional broiler farms (CF), animal welfare-certified broiler farms (AF) need to be equipped with conditions such as low stocking density (less than 19 birds or less than 30 kg/m^2), 2 m length perch for 1,000 broilers, plant-based protein in feeds and environmental enrichments (straw, wood shaving bales, and vegetables). In all these six farms, unsexed chicks (Ross 308) were reared for 28–35 d, and rice hull was used as litter. The size of farms, which is a farm capacity, is 46,000, 200,000, and 120,000 broilers in each CF and 63,000, 108,000, and 180,000 in each AF. The average stocking densities of CF and AF were 19.0 birds/m^2 and 17.9 birds/m^2, respectively. Perch and other enrichments (straw bales, vegetables, etc.) were provided to the broilers, exclusively in the AFs. The lighting program for AF and CF were six h of darkness (from 10:00 p.m. to 4:00 a.m.) and 0 to 4 h of darkness (variously modulated by the farm owners), respectively. The chicks in the CF were fed a diet formulated with 20% crude protein (CP) and 3,000 kcal of apparent metabolizable energy (AME) per kg for starter (0 to 7 d), 19.5% CP, and 3,050 kcal of AME per kg for grower (8 to 21 d), and 17.0% CP and 3,100 kcal of AME per kg for finisher (22 d to the end of the experimental period). The chicks in AF were fed diets formulated with 20.0% CP and 3,000 kcal of AME per kg for starter (0 to 7 d), 19.0% CP with 3,050 kcal of AME per kg for grower (8 to 21 d), and 17.0% CP and 3,100 kcal of AME per kg for finisher (22 d to the end of the experimental period). Only the vegetable ingredients were used as a source of protein in AF, according to guidelines of the Animal and Plant Quarantine Agency (APQA) in Korea. All experimental procedures were approved by the Animal Care and Welfare Committee of the National Institute of Animal Science, Rural Development Administration, Korea (NIAS20191536).

Blood and meat sampling

Thirty chickens from each of the three CFs and three AFs were collected randomly on the day of slaughter (28–35 d) for meat quality analysis. Also, blood was randomly collected from 20 to 30 chickens of each three farms on the slaughter day. The whole-blood samples were taken from the wing vein of the broilers to analyze leukocytes (white blood cells, heterophils, lymphocytes, monocytes, eosinophils, and basophils), erythrocytes (red blood cells, hemoglobin), and serum chemical composition.

After blood sampling, all the breast (*Pectoralis major*) and thigh (*Peroneus longus*) muscles from the broiler were separated after cervical dislocation, evisceration, and washing with water at the farm according to APQA, and then directly

Table 1. Information of the rearing management and slaughtering broilers in conventional and animal welfare-certified farms

Items	Conventional farm				Animal welfare-certified farm			
	1	2	3	Mean	1	2	3	Mean
Farm size	46,000	200,000	120,000	-	63,000	108,000	180,000	-
Housing type	Windowless	Windowless	Open-sided	-	Windowless	Windowless	Windowless	-
Stocking density (birds/m^2)	19.7	19.7	17.6	19.0	17.9	17.9	17.9	17.9
Enrichment materials	----------------------- Not provided -----------------------				Perch, sawdust, cabbage	Perch, sawdust, cabbage	Perch, sawdust, dry corn	-
Slaughter date (low/high temperature)	27 May (14/21℃)	10 June (17/23℃)	26 Aug (18/31℃)	-	15 May (15/29℃)	22 May (12/29℃)	19 June (17/26℃)	-
Slaughter age (day)	30	35	35	33.3	28	33	31	30.6
Slaughter weight (kg)	1.5	1.7	1.8	1.7	1.4	1.7	1.5	1.5

packed with polystyrene trays and wrapped with low-density polyethylene film. The meat samples were stored in a refrigerator at 4°C in the dark and used for analysis at 24 h slaughtering.

Blood analysis

Leucocytes and erythrocytes in blood samples in tubes containing ethylenediaminetetraacetic acid (EDTA, Soyagreentec, Seoul, Korea) were analyzed using the Hemavet Multispecies Hematology Systems (Drew Scientific, Oxford, CT, USA) on the day of blood collection. Serum was separated after centrifugation (20 min, 4°C, 2,500×g) of whole blood and stored at –20°C. The concentrations of total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol, total protein, triglyceride (TG), albumin, glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in serum samples were measured using a blood analyzer (Beckman Coulter AU480®, Brea, CA, USA).

Meat qualities and bioactive compounds

pH

After blending 10 g of meat sample with 90 mL of distilled water for 15 s using a homogenizer (Polytron R PT-2500E, Kinematica, Lucerne, Switzerland), the pH values of the homogenates were determined using an Orion 230A pH meter (Thermo Fisher Scientific, Waltham, MA, USA).

Color

Meat color was measured using a Chroma Meter CR-400 instrument (Minolta, Osaka, Japan). Color values of L* (lightness), a* (redness), and b* (yellowness) were repeatedly measured more than five times. The standard white plate had a Y-value of 93.60, x-value of 0.3134, and y-value of 0.3194.

Water-holding capacity (WHC)

Chicken meat samples (0.5 g) were placed on a round plastic plate in a tube (Millipore Ultrafree MC, Millipore, Bedford, MA, USA), heated in a water bath (20 min, 80°C), cooled to 23±1°C, and then centrifuged (2,000×g, 10 min, 4°C) to measure the water loss. The moisture and crude fat contents were measured according to the AOAC methods (1998), and WHC was calculated using the following equation:

$$\text{WHC (\%)} = (\text{Moisture content} - \text{Water loss}) / \text{Moisture content} \times 100$$

$$\text{Water loss} = (\text{Weight before centrifugation} - \text{Weight after centrifugation}) / (\text{Sample weight} \times \text{Fat factor}) \times 100.$$

$$\text{Fat factor} = 1 - (\text{Crude fat} / 100).$$

Shear force

The meat sample was placed in a polyethylene plastic bag and heated in a constant-temperature water bath (45 min, 75°C) to reach a core temperature of 71°C. After cooling, the samples were cut into 1×2×2 cm pieces. Shear force analysis was performed using a TA 1 Texture Analyzer (Lloyd Instruments, Berwyn, IL, USA). The analyzer was set to a 500 N load cell, 50 mm/min of test speed, 50 mm/min of trigger speed, 10 gf of trigger force and V-shape blade (60° V-notch).

2-Thiobarbituric acid reactive substances (TBARS)

Briefly, 5 g of thigh meat was added to 15 mL distilled water with 50 μL of 7.2% tert-butyl-4-hydroxyanisole (BHA) and homogenized for 30 s (Polytron PT-2500E homogenizer). One mL of the homogenate was then transferred to a test tube, and 2 mL of 20 mM thiobarbituric acid in 15% trichloroacetic acid was added. All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). The sample was heated in a water bath at 90℃ for 15 min, cooled for 10 min, and centrifuged at 2,000×g (for 10 min at 4℃. The absorbance of the supernatant was evaluated at 531 nm using a spectrophotometer (M2e; Molecular Devices, Sunnyvale, CA, USA). The TBARS content was calculated as milligrams of malondialdehyde (MDA) per kilogram of meat using the following equation:

$$\text{TBARS (mg MDA/kg of meat)} = (\text{Absorbance of sample} - \text{Absorbance of the blank sample}) \times 5.88$$

Fatty acid composition

The fatty acid composition of thigh meat samples was measured according to the method described by Kim et al. (2020). Lipid extraction from thigh meat samples was performed with the addition of 20 μL of BHA and 15 mL of a mixture of chloroform and methyl alcohol (2:1, v/v). The homogenates were filtered using filter paper (Whatman No. 1, Maidstone, UK), and the filtrate was vortexed with 3 mL KCl (0.88%) and incubated overnight in the dark (25℃) to separate the two layers. The lower lipid-containing layer was condensed with N_2 (99.9%). A 25 mg lipid sample was mixed with 1.5 mL of 0.5 N NaOH (in methyl alcohol) in glass tubes and heated at 100℃ for 5 min. Then, it was mixed with 1 mL of 10% boron trifluoride and heated at 100℃ for 2 min. After the addition of 2 mL iso-octane and 1 mL saturated NaCl, the samples were centrifuged (783 g for 3 min). Iso-octane extract aliquots were injected into a gas chromatograph (Agilent 6890N Technologies, Santa Clara, CA, USA) equipped with an Omegawax 250 capillary column (30 m×0.25 mm×0.25 μm, Supelco, Bellefonte, PA, USA). The carrier gas, flow rate, and split ratio were helium (99.99%), 1.2 mL/min, and 1:100, respectively. The analytical temperatures of the injector and flame ionization detector were 250℃ and 260℃, respectively. The optimized column temperature program was as follows: initial temperature of 150℃, held for 2 min; a gradual increase in temperature to 220℃ at a rate of 4℃ /min, held at 220℃ for 30 min.

Creatine, creatinine, and dipeptide (anserine and carnosine)

The creatine, creatinine, and dipeptide (carnosine and anserine) contents were measured using the method described by Kim et al. (2020), with slight modifications. Briefly, meat samples (2.5 g) were homogenized with 0.01 N HCl (7.5 mL), and the homogenate was centrifuged at 3,000×g for 30 min. The supernatant was filtered through a glass microfiber filter (GF/C), and 250 μL of the filtrate was deproteinized by incubation with 750 μL of acetonitrile for 20 min at 4℃. The sample was centrifuged at 10,000×g for 10 min, and the supernatant was filtered through a RephiQuik PTFE membrane filter (0.22 μm, RephiLe Bioscience, Shanghai, China) before analysis. The contents of dipeptides (carnosine and anserine) were analyzed using liquid chromatography (1260 Infinity; Agilent Technologies), equipped with an Atlantis HILIC silica column (4.6×150 mm, 3 μm; Waters, Milford, MA, USA) at a column temperature of 35℃. The creatinine content was determined at 236 nm, and creatine and dipeptides (carnosine and anserine) were detected at 214 nm using a diode array detector (Agilent Technologies). Mobile phase A was 0.65 mM ammonium acetate (pH 5.5) in water/acetonitrile (25:75, v/v), and mobile phase B was 4.55 mM ammonium acetate (pH 5.5) in water/acetonitrile (70:30, v/v). We used a linear gradient of phase B from 0% to 100% over 13 min at a flow rate of 1.4 mL/min. The contents were calculated from standard curves generated using standard reagents (Sigma-Aldrich).

Statistical analysis

All data were analyzed by nested analysis of variance using the general linear model univariate procedure of SPSS software (IBM SPSS, version 25, Armonk, NY, USA) in consideration of the effects of different factors during rearing in each farm. The results between means were identified using Duncan's test and were considered significantly different at $p<0.05$.

Results and Discussion

Chemical composition of blood and hematological parameters

The biochemical blood parameters of broilers from the AF and CF groups are shown in Table 2. TC and HDL cholesterol levels between AF and CF did not show any significant differences. However, all of the other serum parameters (LDL-cholesterol, TG, glucose, total protein, and albumin) of AF were significantly lower than those of CF. The serum cholesterol and TG of birds is strongly affected by heredity, nutrition, age, sex, and environmental conditions.

The lower levels of LDL-cholesterol and TG of AF than those of CF in the present study may have resulted from the different conditions of nutrition (using vegetables as a source of protein) and environment (lower stocking density, environmental enrichment, etc.) in AF. The study of Li et al. (2017a) mentioned that plant protein for animal protein decreases the low-density lipoprotein cholesterol apolipoprotein B. We can infer that plant-based proteins affect the reduction of LDL-cholesterol and TG levels in the blood. A comparison of leukocytes according to rearing systems is presented in Table 3. The average white blood cell (WBC) count in the AF group was 14.62 K/μL, which was significantly lower than that of CF (18.60 K/μL; $p<0.001$). All leukocyte values of CF were significantly higher than those of AF ($p<0.001$). The present study showed that the stress index (SI) value of CF (0.43) was significantly higher than that of AF (0.35; $p<0.001$), indicating that broilers are under a moderate range of stress. This lower SI value might be affected by the low stocking density and perch

Table 2. Comparison of serum chemical composition of broiler reared in conventional and animal welfare-certified farms

Serum composition	Conventional farm			Animal welfare-certified farm			Mean		SEM	p-value
	1 (n=22)	2 (n=22)	3 (n=20)	1 (n=25)	2 (n=25)	3 (n=30)	Conventional farm	Animal welfare-certified farm		
TC (mg/dL)	109.22	100.15	128.91	120.82	98.92	102.32	112.76	107.35	1.942	0.127
HDL (mg/dL)	105.87	90.07	113.43	113.26	96.77	104.51	103.12	104.83	1.503	0.414
LDL (mg/dL)	72.15	63.28	67.37	73.28	59.24	54.09	67.62[a]	62.25[b]	1.271	<0.050
TG (g/dL)	34.14	60.99	80.31	43.24	45.23	40.04	58.47[a]	42.84[b]	1.863	<0.001
Glucose (mg/dL)	168.49	189.81	181.29	199.69	120.29	160.06	179.86[a]	160.01[b]	4.215	<0.010
TP (g/dL)	3.58	3.76	3.47	3.16	3.57	2.69	3.60[a]	3.14[b]	0.055	<0.001
Albumin (g/dL)	1.08	1.07	1.12	1.08	1.06	0.95	1.09[a]	1.03[b]	0.012	<0.010
AST (U/dL)	360.12	352.75	339.61	406.02	374.94	342.59	350.83	374.52	6.705	0.075
ALT (U/dL)	2.44	2.69	1.73	2.79	2.18	2.68	2.29[b]	2.55[a]	0.052	<0.010

[a,b] Means with different superscripts between conventional and animal welfare-certified farms are significantly different at $p<0.05$.
TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglyceride; TP, total protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 3. Comparison of the hematological profile of broiler reared in conventional and animal welfare-certified farms

Hematological profile	Conventional farm			Animal welfare-certified farm			Mean		SEM	p-value
	1 (n=22)	2 (n=22)	3 (n=20)	1 (n=25)	2 (n=25)	3 (n=30)	Conventional farm	Animal welfare-certified farm		
WBC (K/μL)	18.54	19.85	17.42	12.66	18.40	12.81	18.60[a]	14.62[b]	0.404	<0.001
HE (K/μL)	4.77	5.11	4.72	2.77	4.59	2.95	4.87[a]	3.44[b]	0.145	<0.001
LY (K/μL)	11.18	11.92	10.12	8.31	11.28	8.22	11.07[a]	9.27[b]	0.195	<0.001
SI (HE/LY)	0.42	0.42	0.46	0.31	0.40	0.35	0.43[a]	0.35[b]	0.008	<0.001
EO (K/μL)	0.52	0.60	0.61	0.28	0.52	0.29	0.58[a]	0.36[b]	0.045	<0.001
MO (K/μL)	1.93	2.06	1.81	1.29	1.89	1.30	1.93[a]	1.49[b]	0.022	<0.001
BA (K/μL)	0.14	0.16	0.16	0.06	0.12	0.05	0.15[a]	0.08[b]	0.008	<0.001
RBC (M/μL)	2.30	2.30	2.20	2.20	2.40	2.10	2.34	2.31	0.018	0.274
Hb (g/dL)	8.60	8.10	7.30	8.10	11.40	7.50	8.09	9.03	0.497	0.249

[a,b] Means with different superscripts between conventional and animal welfare-certified farms are significantly different at p<0.05.
WBC, white blood cells; HE, heterophils; LY, lymphocytes; SI, Stress Index; EO, eosinophils; MO, monocytes; BA, basophils; RBC, red blood cell; Hb, hemoglobin.

installation in AF. Onbasilar et al. (2008) reported that the H/L ratio was higher in broilers reared at high stocking density (17.5 birds/m^2) than in broilers reared at low stocking density (11.9 birds/m^2; p<0.001), and Campo et al. (2005) demonstrated that the SI value of hens reared with perches was lower than that of hens reared without perches.

Meat quality properties

The effects of the rearing system on breast and thigh meat quality are shown in Table 4. Generally, the pH of fresh chicken meat is approximately 5.3–6.5 (Hertanto et al., 2018). The pH of breast meat from CF was significantly lower than that of AF (p<0.001). This result was inconsistent with the results of Kim et al. (2020), who reported no difference between the two rearing systems. Unlike breast meat, the thigh meat pH showed no difference. This result is in contrast to those of Kim et al. (2018) and Castellini et al. (2002), who showed a significantly lower level of thigh meat pH in AF. Castellini et al. (2002) reported that organic farming reduced the pH of meat because the low-stress levels in the welfare environment have diminished consumption of glycogen in muscles. Therefore, further research on the extent of activities that affect pH values is necessary in broilers.

In Table 4, L*, a*, and b* of breast meat had no difference between the two rearing systems. These results are quite different from the results of USDA (2011) that exercised chickens are darker than non-exercised chickens in meat color, which means that the L* value is low. There was also no difference in the WHC of breast meat between the two rearing systems. In thigh meat, the average values of L*, a*, b*, and WHC also showed no difference between the two rearing systems.

The average shear force of breast meat from AF (28.31 N) was significantly higher than that of CF (23.47 N; p<0.001), which is consistent with the results of Castellini et al. (2002). They reported that shear force, an indicator of meat tenderness, increased as the activity of broilers increased. Li et al. (2017b) also reported that the shear force of breast meat was significantly different depending on the rearing system. They also reported that chickens in the cage group showed

Table 4. Meat pH, instrumental color, water holding capacity, cooking loss, and shear force of broilers from conventional and animal welfare-certified farms

Items		Conventional farm			Animal welfare-certified farm			Mean		SEM	p-value
		1	2	3	1	2	3	Conventional farm	Animal welfare-certified farm		
Breast meat											
pH		5.90	5.89	5.97	6.03	6.05	6.03	5.92[b]	6.04[a]	0.018	<0.001
Color	L*	56.24	54.91	54.32	55.74	57.28	52.67	55.16	55.23	0.378	0.902
	a*	1.90	1.81	1.64	1.91	1.92	1.57	1.78	1.80	0.073	0.916
	b*	6.23	6.39	7.81	6.65	6.26	9.42	6.81	7.44	0.270	0.092
WHC (%)		50.48	50.04	50.76	46.79	54.12	50.93	50.43	50.61	0.728	0.888
Cooking loss (%)		17.78	18.76	18.75	18.56	16.80	17.59	18.43	17.65	0.322	0.238
Shear force (N)		22.32	24.30	23.78	31.86	26.53	26.55	23.47[b]	28.31[a]	0.630	<0.001
Thigh meat											
pH		6.39	6.30	6.30	6.36	6.30	6.35	6.33	6.34	0.014	0.725
Color	L*	54.33	56.25	55.01	56.32	55.39	54.85	55.20	55.52	0.367	0.671
	a*	8.23	7.73	6.59	6.99	8.32	7.44	7.52	7.58	0.169	0.806
	b*	7.36	8.34	9.43	7.25	7.30	12.32	8.38	8.96	0.374	0.123
WHC (%)		48.78	49.29	52.40	53.37	49.44	51.73	50.15	51.51	0.640	0.274
Cooking loss (%)		24.58	29.72	27.64	25.70	24.73	25.62	27.31[a]	25.35[b]	0.535	<0.050
Shear force (N)		22.26	25.29	24.09	26.03	24.76	25.60	23.88[b]	25.46[a]	0.381	<0.050

[a,b] Means with different superscripts between conventional and animal welfare-certified farms are significantly different at $p<0.05$.
WHC, water holding capacity.

significantly lower shear force than chickens in the indoor-floor group ($p<0.05$).

The value of the shear force of thigh meat was also significantly higher in AF (25.46 N) than in CF (23.88 N; $p<0.05$), as it was in the case of breast meat. It can be assumed that broilers reared in a lower density of AF had more activity, leading to an increased shear force.

2-Thiobarbituric acid reactive substances (TBARS) and total cholesterol

TBARS values of thigh meat are a common way to measure lipid oxidation. TBARS of thigh meat and TC of the thigh skin are shown in Table 5. In TBARS, there was no statistically significant difference between the rearing systems. The average cholesterol level in the thigh skin was not significant; however, the cholesterol level of AF (93.9 mg/100 g) tended to be lower than that of CF (104.08 mg/100 g).

Although the TC value of thigh skin showed no statistical significance due to the small number of samples, the low cholesterol level is expected to serve as a value-added factor for modern consumers who are more interested in health. Dietary cholesterol itself does not lead to increased cholesterol levels in the body; however, foods containing high levels of cholesterol tend to contain high levels of saturated and other unhealthy fats.

Table 5. 2-Thiobarbituric acid reactive substance and total cholesterol of thigh meat of broilers from conventional and animal welfare-certified farms

Items	Conventional farm			Animal welfare-certified farm			Mean		SEM	p-value
	1	2	3	1	2	3	Conventional farm	Animal welfare-certified farm		
TBARS (mg MDA /kg)	0.16	0.17	0.17	0.18	0.17	0.14	0.17	0.16	0.003	0.255
Cholesterol in chicken thigh skin (mg/100 g)	108.50	110.95	92.80	91.35	87.50	102.85	104.08	93.90	3.988	0.237

TBARS, 2-Thiobarbituric acid reactive substances; MDA, malondialdehyde.

Fatty acid composition

AF are fed diets containing plant ingredients, such as soybeans, canola, peas, and sunflowers, instead of animal resources for protein compounds. In the present study, the values of stearic acid, vaccenic acid, γ-linogenic acid, eicosenoic acid, arachidonic acid, adrenic acid, and docosahexaenoic acid showed no difference between the two rearing systems (Table 6). However, palmitic acid (C16:0), palmitoleic acid (C16:1n7), and oleic acid (C18:1n9) were significantly higher in CF. On the other hand, myristic acid (C14:0), linoleic acid (LA, C18:2n6), α-linolenic acid (ALA, C18:3n3), and eicosapentaenoic acid (EPA, C20:5n3) was significantly higher in AF than in CF.

The value of PUFAs in thigh meat was significantly higher in AF than in CF ($p<0.001$). In PUFAs, the ALA and EPA values of w-3 PUFAs and LA values of w-6 PUFAs were high. Omega-3 PUFAs have been shown to have anti-inflammatory and properties (Oppedisano et al., 2020), whereas w-6 PUFAs generally act as pro-inflammatory (Berger et al., 2017).

These higher values of the w-3 PUFAs (ALA, EPA) in AF ($p<0.001$) may have come from the plant-based protein in the diet, according to the report of Healthline Nutrition (2017). They reported that the ALA is the most common fatty acid found in plant-based foods and is converted to the long chain omega-3 EPA and DHA.

Rustan and Drevon (2005) reported that dietary marine w-3 FAs (EPA and DHA) decreased plasma triacylglycerol levels by reducing the production of triacylglycerol-rich lipoproteins (inhibiting lipogenesis) and that w-6 PUFAs were inversely correlated with plasma TG. Therefore, high values of dietary EPA and w-6 PUFAs in AF may have contributed to the lowering of TGs in the blood (Table 2).

Chicken meat with high LA and ALA can be a good source of essential fatty acids in humans. It has been reported that a high w-6/w-3 ratio is thought to have adverse health effects (Berger et al., 2017). The w-6/w-3 ratio of AF (21.02) was significantly higher than that of CF (19.50; $p<0.001$). The guideline of the PUFA/SFA ratio of human diets is above 0.45 and the w-6/w-3 ratio is under 4.0 (Ribeiro et al., 2013). The PUFA/SFA ratio meets this requirement, while the w-6/w-3 ratio is higher than the criteria in both rearing systems. Further studies are needed to modulate the w-6/w-3 ratio in the muscle of broilers from AF.

Creatine, creatinine, and dipeptide (anserine and carnosine) content

Creatine, non-essential amino acid, is known to provide energy to the muscles and protect nerves (Kim et al., 2018) and is interchangeable with creatinine at a constant rate.

The average value of the creatine of breast meat in AF was 328.40, significantly lower than that of CF (346.56 mg/100 g;

Table 6. Fatty acid composition of thigh meat of broilers reared under conventional and animal welfare-certified farms

Fatty acid (%)	Conventional farm			Animal welfare-certified farm			Mean		SEM	p-value
	1	2	3	1	2	3	Conventional farm	Animal welfare-certified farm		
C14:0	0.77	0.87	0.71	0.87	0.88	0.87	0.78[b]	0.87[a]	0.013	<0.001
C16:0	24.09	23.13	23.18	23.33	23.20	22.79	23.47[a]	23.11[b]	0.098	<0.010
C16:1n7	6.05	4.98	5.81	5.49	4.93	4.89	5.61[a]	5.10[b]	0.115	<0.001
C18:0	6.76	7.20	6.72	6.63	7.09	6.65	6.89	6.79	0.071	0.632
C18:1n9	40.78	39.01	39.62	39.77	39.65	37.68	39.80[a]	39.03[b]	0.183	<0.001
C18:1n7	2.42	2.27	2.39	2.40	2.27	2.39	2.36	2.35	0.016	0.931
C18:2n6	16.26	19.37	18.10	18.26	18.68	20.84	17.91[b]	19.26[a]	0.275	<0.001
C18:3n6	0.24	0.22	0.27	0.26	0.22	0.24	0.24	0.24	0.004	0.447
C18:3n-3	0.73	1.21	0.86	1.11	1.14	1.17	0.93[b]	1.14[a]	0.034	<0.001
C20:1n9	0.47	0.42	0.47	0.45	0.44	0.46	0.45	0.45	0.005	0.555
C20:4n-6	0.93	0.90	1.30	0.94	0.90	1.37	1.04	1.07	0.045	0.467
C20:5n-3	0.11	0.08	0.08	0.11	0.17	0.15	0.09[b]	0.14[a]	0.009	<0.050
C22:4n6	0.24	0.22	0.33	0.24	0.28	0.35	0.26	0.29	0.013	0.302
C22:6n-3	0.13	0.11	0.15	0.14	0.15	0.15	0.13	0.15	0.005	0.191
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	-	-
SFA	31.62	31.20	30.61	30.83	31.17	30.31	31.14[a]	30.77[b]	0.105	<0.050
USFA	68.38	68.80	69.39	69.17	68.83	69.69	68.86[b]	69.23[a]	0.105	<0.050
MUFA	49.72	46.68	48.29	48.11	47.29	45.42	48.23[a]	46.94[b]	0.275	<0.001
PUFA	18.66	22.12	21.09	21.06	21.54	24.27	20.62[b]	22.29[a]	0.327	<0.001
MUFA/SFA	1.57	1.50	1.58	1.56	1.52	1.50	1.55	1.53	0.009	0.090
PUFA/SFA	0.59	0.71	0.69	0.68	0.69	0.80	0.66[b]	0.72[a]	0.012	<0.001
ω-6/ω-3	18.01	20.87	19.62	19.93	20.37	22.77	19.50[b]	21.02[a]	0.293	<0.001

[a,b] Means with different superscripts between conventional and animal welfare-certified farms are significantly different at p<0.05.
SFA, saturated fatty acid; USFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

p<0.01), and the mean creatinine content between AF and CF was not different (Table 7). These results are inconsistent with the report of Wyss and Kaddurah-Daouk (2000), who suggested that exercise facilitates the absorption of creatine in the muscles, and Kim et al. (2020) reported no difference in creatine and creatinine values between the broilers reared in the two rearing systems. In Table 7, the average creatine value of thigh meat in AF before catching (337.50 mg/100 g) was significantly lower than that of CF (354.45 mg/100 g; p<0.05), which contrasts with the study. The average creatinine content of thigh meat in AF was 3.03 mg/100 g, which was significantly higher than that of CF (2.59 mg/100 g; p<0.001).

It can be assumed that chickens, one of the burst-like animals (Derave et al., 2010), reared in AF had opportunities to perform more activities such as walking and moving around, perching, wing-flapping, pecking (Australian Animal Welfare Standards and Guidelines, 2018), resulting in elevated levels of creatinine.

Recent studies have highlighted the essential role of the endogenous bioactive compounds such as carnosine, anserine of meat in human health (Jayasena et al., 2014). Anserine and carnosine are well known as antioxidants that prevent diseases

Table 7. Creatine, creatinine, and dipeptides contents of broilers from conventional and animal welfare-certified farms

Items (mg/100 g)	Conventional farm			Animal welfare-certified farm			Mean		SEM	p-value
	1	2	3	1	2	3	Conventional farm	Animal welfare-certified farm		
Breast meat										
Creatine	326.57	327.04	386.04	308.50	329.94	346.74	346.56[a]	328.40[b]	5.232	<0.010
Creatinine	2.69	2.62	2.10	3.32	2.56	2.15	2.47	2.68	0.090	0.077
Anserine	184.71	231.66	242.82	225.72	251.14	226.43	219.73	234.43	5.897	0.144
Carnosine	59.25	73.04	77.54	79.71	82.62	90.56	69.94[b]	84.30[a]	2.429	<0.001
Thigh meat										
Creatine	330.73	342.29	390.33	309.57	332.78	370.14	354.45[a]	337.50[b]	5.825	<0.050
Creatinine	2.59	3.02	2.16	2.57	3.48	3.03	2.59[b]	3.03[a]	0.092	<0.001
Anserine	159.84	151.09	123.60	123.53	157.46	112.76	144.84[a]	131.25[b]	3.816	<0.001
Carnosine	48.15	55.65	72.21	59.76	60.12	73.24	58.67[b]	64.37[a]	2.003	<0.050

[a,b] Means with different superscripts between conventional and animal welfare-certified farms are significantly different at p<0.05.

caused by fat oxidation in skeletal muscle (Kim et al., 2012). Anserine has the buffering capacity and antioxidant properties (Jayasena et al., 2014), and the amount of anserine may decrease due to a nutritional disorder in the muscles (Kim et al., 2018). Carnosine is a naturally occurring histidyl dipeptide (*N*-β-alanyl-L-histidine) with several biological functions. Jayasena et al. (2014) reported its potential buffering role in skeletal muscle, antioxidant (O'Neill et al., 1999), and anti-aging properties, and carnosine aids muscle recovery from exercise.

The average value of anserine of breast meat was not different by the rearing conditions, but that of thigh meat was significantly lower in AF (131.25 mg/100 g) than that of CF (144.48 mg/100 g; p<0.001). The average values of carnosine in breast and thigh meat from AF were significantly higher than those from CF (Table 7).

Juniper and Rymer (2018) reported that the increase in anserine level in breast meat may have been contributed by the higher amount of exercise when broilers were reared in the free-range system providing more space to move. Anserine in the breast meat was not significant but tends to increase in broilers reared in AF providing more space allowing more physical activity than that in CF. A study by Derave et al. (2010) supported the results of our study, it is known that animals related to the more explosive-like and intense exercise such as chickens, greyhound dogs, thoroughbred horses have prominently higher concentrations of histidine-containing dipeptide such as carnosine. More research is needed due to the lack of research on bioactive compounds of chicken meat.

Conclusion

The beneficial effects of the AF system on broilers were demonstrated by the low levels of LDL-cholesterol, TG, and SI compared to those levels in CF. In the meat quality, shear force and carnosine levels in breast and thigh meat of broilers reared in AF were significantly higher than those of CF. There were also significant increases in PUFA of thigh meat such as LA and ALA, which are good sources of essential fatty acids in poultry and humans. These results can be used as preliminary data indicating chicken meat produced from AF at the farm level before transportation. Further studies are needed to enhance the accuracy of the effect of the animal welfare system and to identify characteristics that can be used as an indicator for chicken meat from animal welfare certified farms.

Conflict of Interest

The authors declare no potential conflict of interest.

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Author Contributions

Conceptualization: Jeon JJ, Kim SH. Data curation: Jeon JJ, Jang A. Formal analysis: Jeon JJ, Kang HK, Kim CH. Methodology: Jeon JJ, Kim SH. Validation: Jeon JJ, Kim Hee-Jin. Investigation: Jeon JJ, Kim HS, Kim SH, Kang BS. Writing - original draft: Jeon JJ. Writing - review & editing: Jeon JJ, Kim Hee-Jin, Kim Hye-Jin, Kang HK, Kim CH, Kim HS, Kang BS, Kim SH, Jang A.

Ethics Approval

The protocol for this experiment was approved by the Institutional Animal Care and Use Committee of National Institute of Animal Science, Rural Development Administration, Korea (NIAS20191536).

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