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Applicability of non-invasive, digital palpation device to detection of woody breast conditions in chicken breast muscle

ABSTRACT

Woody Breast (WB) is one of muscle myopathy found in chicken breast, characterized with enlarged size and extremely stiff texture. The WB condition is one of the most prevalent quality issues in the modern poultry industry. WB has been shown to be heritable, but no effective detection method of WB severity in live birds exists for the selection purpose. The objective of this study was to determine potential of a non-invasive, portable digital palpation device as WB detection method that can be used for the selection to estimate the heritability of WB. The physical and functional properties of WB was also investigated in comparison with normal breast (NB). Two hundred ten breast muscles were obtained from a local processing plant one day after harvest and sorted based on WB scoring (1 for NB and 2 and 3 for WB). The samples were subjected to physical and physicochemical analyses, determining biomechanical properties (muscle tone, stiffness, elasticity, relaxation, and creep), pH, color, cooking yield, and texture (firmness and compression energy were used for raw meat and shear force and energy for cooked meat). The least squares means of the following variables were significantly different between WB and NB ($p<0.01$): stiffness (603.4 vs 565.8; N/m), and elasticity (1.40 vs 1.55). However, relaxation and creep were not significantly different ($p>0.05$). These results collectively showed that biomechanical properties of WB differ from NB. The degree of muscle stiffness in WB can be considered as a trait to be selected. The WB score showed strong negative correlations with cooking yield (-0.77) and cooked L*(-0.74), which means that as the breast becomes harder, the cooking yield decreases, and the color becomes darker after cooking. The WB score showed high correlations with physical and functional characteristics and exhibited strong correlations with the biomechanical properties measured by the device.

Therefore, the results indicated that the digital palpation device has potential to detect the WB severity (degree of stiffness) of breast muscle.

Keywords: woody breast, Myoton, characteristics, broiler, breast meat

INTRODUCTION

Poultry producers' main goal is to raise chickens as fast and large as possible. This quest for growth efficiency is thought to have caused many muscle abnormalities, and a woody breast (WB) is one of them that is a significant concern for numerous producers and consumers in countries throughout the world, yet no practical solution has been proposed [1,2].

"Woody Breast" is the broiler breast muscle abnormality featured with enlarged size and extremely stiff textural properties often accompanies by hemorrhages on the surface, causing quality issues of broiler breast meat [2]. The WB condition is one of the most prevalent issues in the modern poultry industry. The frequency of the defect in Europe is over 30% and some have argued that similar levels may exist in the US, depending on the weight of the bird at harvest [3,4]. Given that chicken with WB is of poor economic value and are discriminated by the consumers, producers face significant economic hardship under the current production paradigm as the producers can find out occurrences of WB only after harvest [5-9].

One of the problems in identifying a solution is that the occurrence and severity of WB can be identified only after harvest. More specifically, WB is subjectively evaluated and separated by meat processors using visual cues and manual palpation after harvest [10]. Because more and more wholesale and retail vendors discriminate against WB chicken, WB is becoming a huge economic liability for the poultry industry [6,9]. As a result, defining and selecting against this quality defect is absolutely necessary for the future health of the poultry industry, specifically chicken (broiler) production.

Despite its largely unknown etiology, genetics, especially higher growth rate and breast muscle yield, are believed to be closely associated with the incidence and severity of WB [1,11-13]. Studies have indicated that WB shows moderate heritability (h^2 = 0.1 - 0.49) [14,15]. Therefore, if WB is heritable and measurable before harvest, it means that it can be controlled by genes to certain extent and therefore can be reduced by animal breeding – selection and mating. Genetic selection is a method to estimate the genetic (breeding) values of individual animals with pedigree information and environmental factors. Breeding value is the value of an individual as a (genetic) parent in a breeding program for a specific trait [16]. Bailey et al. [17] showed that the incidence of WB was reduced by 18.4% after 2 years of the genetic selection process.

Based on the previous studies, we hypothesized in this study that WB is heritable and therefore it can be selected for, based on the severity of WB at certain point of growth of live birds. In order to successfully carry out the effective selection, we need to know the degrees to which the severity of WB could be adequately measured in live birds and quantified as a phenotype. Then the phenotype can be subjected to a selection study in the area of animal breeding using genetic (pedigree) information and quantitative (statistical) genetics.

However, the major obstacle against animal breeding research for the solutions of WB is an absence in an effective, reliable detection system of WB in the breast tissues of live birds along with an accurate, quick method to evaluate the severity of WB, which would be considered as the level of hardness. There have been advancements in this area. Tijare et al. [18] described the WB scoring method with regard to level of hardness and location (cranial vs. entire fillet) and Mudalal et al. [19] used texture analysis measuring the force required to compress fillets to determine the WB severity of chicken breasts. While those evaluations methods for textural characterization of WB after harvest are useful for separating WB from normal breast, they cannot be used for the detection of WB and its severity in affected muscle tissue of live birds for animal breeding.

Myotonometry is a non-invasive technique that can measure biomechanical characteristics of muscle tone including stiffness by applying mechanical force perpendicularly to the muscle tissues [20]. Validity, reliability, and convenience of hand-held myotonometer measuring muscle stiffness in vivo have been demonstrated by previous clinical studies [20-22]. This demonstrates that the portable myotonometer can be used to measure WB in live birds. The objective of this study was to determine the applicability of myotonometer, a non-invasive, hand-held, digital palpation device, to the quantification of the severity of WB as a trait that can be used for the selection study to estimate the heritability of WB. This study also determines the physical and functional characteristics of WB in comparison with normal breast (NB).

MATERIALS AND METHODS

Sample preparation

Two hundred ten chicken breast fillets (*Pectoralis major*) with woody and normal conditions (35 WB and 35 NB per replication, 3 replications) were obtained from a local processing plant one day after slaughtering. The breast fillets were sorted into woody and normal conditions at the processing plant based on the plant processing procedure. Up on receipt, the severity of woody breast conditions of the samples was evaluated based on the WB scoring system described by Tijare et al. [18] with some modifications: 1 – normal (flexible throughout), 2 – moderate (rigid mainly in the cranial region but flexible in the caudal region), and 3 – severe (extremely rigid throughout the breast meat). They were divided into 2 groups based on the WB scoring: NB - 1 and WB - 2 and 3. Subsequently, the fillets were prepared for the evaluation of physical, physicochemical, and functional properties, including pH, color, water holding capacity, cooking yield, and textural properties. All the assays were completed within

2 days after the receipt.

Biomechanical properties of raw breast muscle

Biomechanical properties of the samples were determined using a myotonometer, non-invasive, portable digital palpation device (MyotonPRO, Myoton, Tallinn, Estonia). The probe of the device was placed perpendicular to the cranial portion of the breast muscle and then the mechanical impulse was briefly applied to the muscle at a force of 0.60 N for 15 ms [23]. The resulting oscillation curve of the muscle were recorded and used to calculate 5 biomechanical properties of muscle, including muscle tone, stiffness, elasticity, relaxation, and creep. Those properties were defined as follows: 1) muscle tone, intrinsic tension in the relaxed muscle measured as oscillation frequency (Hz), 2) stiffness, the resistance of muscle to deformation force measured as dynamic stiffness (N/m), 3) elasticity, the ability to recover its initial shape after removal of an external force of deformation measured as logarithmic decrement, 4) relaxation, the time for muscle to recover its shape from deformation after the removal of an external force measured as mechanical stress relaxation time (ms, MSRT), and 5) creep, the gradual elongation of a tissue over time when placed under a constant tensile stress measured as ratio of deformation and relaxation time (RDRT) [20,24,25].

pH and color

The pH of each breast fillet was measured using a portable pH meter (HI98163, Hanna Instrument, Smithfield, RI) equipped with an insertion pH electrode (FC2323). The electrode tip was inserted into the fillet approximately 1 cm below the surface and the reading was recorded after stabilized. Color on the skin-side surface of the fillet was determined using a colorimeter (CR-400, Konica Minolta Sensing America, Ramsey, NJ, USA) with 8 mm aperture, illuminant D65, and 2° standard observer. The color values taken from 3 random locations in the cranial region of each fillet were averaged and reported. The color of the cooked breast fillet was also determined.

Water holding capacity

The water holding capacity (WHC) of the raw fillet sample was determined using a centrifugation method [26]. A cell strainer containing a thin slice of breast fillet (approximately 1 g and 1-2 mm in thickness) was placed onto a tube and centrifuged at 400 × g for 1 hr at 4 °C to remove free and loosely bound water from the meat. WHC (%) was calculated as the ratio of the weight of water removed to the initial weight of the slice and represented the amount of removable water (water loss) in 100 g of meat.

Cooking yield

A portion of breast fillet including the cranial region (approximately 200 g) was vacuum-packaged and cooked in a boiling water bath until the internal temperature reached 73.9 °C. After excessive moisture and fat on the surface, the cooked sample was stored at 4 °C in a refrigerator overnight prior to weighing. Cooking yield (%) was calculated as a ratio of the weight of the sample after and before cooking and expressed as a percentage. Subsequently, the cooked meat was used to determine the shear force and energy.

Firmness of raw breast fillet

The firmness and compression energy of the raw breast fillet sample were determined in the cranial region using a Texture analyzer (model TA-XT2i, Texture Technologies, Hamilton, MA) equipped with a 50-kg loading cell and a cylinder probe (TA-10ss, 12.5 mm in diameter) attached to a converter (TA-71). The cranial region of the samples was compressed 10 mm in depth by the probe at the crosshead speed of 10 mm/sec. Peak force (N) and area under the time-force curve (N*sec) were reported as firmness and compression energy.

Shear force of cooked breast meat

Shear force and energy of the cooked breast meat were determined using the Meullenet-Owens Razor Shear (MORS) method [27], using a Texture analyzer equipped with a 5-kg loading cell and MORS blade (TA-46, 9 mm (blade) x 38 mm). The cooked breast meat was penetrated by the MORS blade perpendicular to the fiber direction. The penetration depth and crosshead speed were 20 mm and 10 mm/sec, respectively. The peak force (N) and area under the time-force curve (N*mm) were calculated and reported as the shear force and energy, respectively.

RESULTS

Table 1 shows the significance of factors included in the statistical model. The effects of test and breast type were significant in almost all dependent variables except for water loss, b* and frequency. The interaction effect was generally not significant, but it was significant in some cases and was not excluded from the statistical model.

Least square means of physical and physicochemical characteristics are shown in Table 2. The least squares means of the following variables were significantly different between WB and NB ($p<0.01$): DS (603.4 vs 565.8; N/m), and elasticity (1.40 vs 1.55). However, MSRT and RDRT were not significantly different ($p>0.05$). These results collectively show that the non-invasive measurements of WB differ from NB. The degree of muscle hardness in WB can be considered as a trait to be selected, and utilized for the genetic/genomic selection program collecting the measurements before harvest.

Pearson correlations are shown among parameters studied in Table 3. The WB score showed strong negative correlations with cooking yield (-0.77) and cooked L*(-0.74), which means that as the breast becomes harder, the cooking yield decreases, and the color becomes darker after cooking. The WB score showed high correlations with physicochemical characteristics and also exhibited strong correlations with the values measured by the device used in this study. Therefore, it was possible to measure the hardness of breast meat using the device and also observe the presence of variability.

DISCUSSION

There are large amounts of research going on in the industry to try and improve meat quality without decreasing the performance of the bird or negatively impacting the poultry industry and human health [6]. To date, the biological mechanism responsible for WB remains unknown [28]. About 90 studies were found in PubMed regarding WB, of which 34% were done on how it affected the different qualities of the meat; 23% on how the feeding regimen/diet affected the incidence or severity of the meat; 15.5% on how different genetic lines affected the incidence of the condition and which genes were expressed due to the condition; about 18% on the histology and morphology of the affected meat; 5.5% on the pathology; 2% on the incidence of the condition is affected by the age of the bird; and just 1% on how the time of hatch and incubation temperature can affect the morphology score [29].

Although WB poses trouble for poultry industries across the globe, there still hasn't been a practical solution or set of policies that are proposed or in use today because of limited research and information on it [30]. Woody breast is a phenomenon that affects the physical composition of broiler raw breast fillets. It has been reported that WB leads to multiple histological lesions such as myodegeneration and necrosis and regenerative changes [31,32].

Over the last 10 years, continuous selection for broilers resulted in about a 5% increase in breast meat yield [32]. This selection effort to increase bird size has led to a higher risk of disease incidences, economic loss, and welfare concerns, as well as a negative influence on meat quality traits [33,34]. Increased growth rate and continuous selection saw various muscular defects [35,36] and increased muscle damage in chickens [37]. Furthermore, heavy broilers under intensive selection also had higher rates of myodegeneration and diminished thermoregulatory capacity, altered cation regulation in muscle cells and more resulting in various meat quality defects [32,38]. Why increased growth rates in modern broilers causes such myopathies is not known, but some researchers reported that heavier birds have higher incidence of severe WB [1,11-13]. Another consequence the WB phenomenon brings about is severe economic loss. As breast meat is a widely sought out source of lean meat across the nation, changes in the composition and anatomy of the breast translates to degradation of meat quality, texture, nutrition and taste, a devastating blow to the domestic poultry industry [9].

There have been some reports regarding hereditary muscular dystrophy in domestic fowl [39], in which affected birds exhibit a broad shallow body and short thick limb bones [40]. Histopathological studies saw wide variations in fiber size, fast deposition, degeneration of the muscle fibers and more [41,42]. On top of changes in appearance, tenderness and fat content were also influenced [43,44]. Hereditary muscular dystrophy shares histological lesions with WB. Hete and Shung [45] observed that the tissue of WB chicken was stiff and had a rubbery texture compared to their control lines with flaccid muscles. There have also been approaches to studying gene expressions of WB meat.

Velleman [46] studied gene expressions of WB affected meat and reported that different broiler lines in the study possessed different cellular mechanisms. Lack of these nutrients could damage cells and retard integral cellular reactions and processes, raising the influence and occurrence of harmful pathological conditions. Moreover, increased selection process and higher breast yield could be the source behind the various myopathies, according to Petracci et al. [32]. This statement was highlighted in Bailey et al. [14] when they observed two different lines of broiler chicken and the chicken with higher breast yield showed a greater incidence in myopathies than the chicken with lower breast yield.

Challenge regarding WB research is an absence of an effective standardized scoring scale since recording and judging WB relies on a subjective scoring system. When scoring, having multiple people as opposed to one person reaching a consensus on the severity of WS and WB may be more effective until scorers are familiar with the existing or upcoming scoring systems. Fast methods to evaluate hardness would also be helpful. There have been some advancements in this area, where Tijare et al. [18] described scoring methods with regard to level of hardness and location (cranial vs. entire fillet) and Mudalal et al. [19] used texture analysis to determine the force required to compress fillets, thus determining the severity of the physical state of chicken breasts with WB.

CONCLUSION

In this study, WB was found to be measurable with a device and it has a variation, which means that chickens would be selected based on the severity of WB. We knew that the severity in the symptom of WB (meat) could be scored (quantified) as a phenotype. The next stage of the study will score the parameters of breast muscle during the growth of broilers (live birds) to assess the exact time or time interval of when WB can be detected and indexed to pinpoint the onset of WB in growing broilers. Each individual can be scored based on its severity, and the scores will be used to select the chickens to be mated. In sum, a selection study will be possible with this proposed study to establish a genetic line to minimize the severity of WB while maximizing the growth rate and all other economically important traits using genetic (pedigree) information and quantitative genetics (statistical animal breeding) [47].

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Table 1. Significance of factors included in the statistical model.

	Test	Breast Type	Interaction
pH	**	**	NS
Cooking Yield	**	**	**
Water Loss	NS	**	NS
Raw L^*	**	**	NS
Raw a^*	NS	**	NS
Raw b^*	**	**	**
Cooked L	**	**	**
Cooked a	*	**	NS
Cooked b	**	**	NS
Firmness	**	**	**
Work Penetration	**	**	**
Shear Force	**	**	**
Shear Energy	**	**	**
Muscle tone	**	NS	NS
Stiffness	**	**	NS
Elasticity	**	**	NS
Relaxation	**	**	NS
Creep	**	**	NS

** $p < 0.01$; * $p < 0.05$; NS: Not Significant

Table 2. Least square means of physical, physicochemical, and functional characteristics.

	Test 1		Test 2		Test 3	
	Normal	Wooden	Normal	Wooden	Normal	Wooden
pH	6.00±0.02^{a} (30)	6.12±0.01^{b} (33)	5.79±0.01^{a} (35)	5.96±0.01^{b} (34)	5.76±0.01^{a} (33)	5.92±0.01^{b} (31)
Cooking Yield (%)	73.5±0.46^{a} (31)	60.2±0.43^{b} (35)	72.3±0.45^{a} (33)	66.7±0.44^{b} (34)	69.9±0.44^{a} (34)	59.5±0.43^{b} (35)
Water Loss (%)	9.22±0.46^{a} (35)	11.8±0.46^{b} (35)	9.95±0.46^{a} (35)	11.7±0.48^{b} (32)	9.57±0.46^{a} (35)	11.9±0.47^{b} (33)
Raw L^*	63.3±0.38^{a} (35)	66.2±0.38^{b} (34)	61.9±0.38^{a} (35)	65.9±0.38^{b} (34)	61.4±0.39^{a} (33)	64.6±0.38^{b} (35)
Raw a^*	0.73±0.14^{a} (34)	1.56±0.14^{b} (33)	0.89±0.14^{a} (34)	1.35±0.13^{b} (35)	0.55±0.14^{a} (31)	1.67±0.13^{b} (35)
Raw b^*	6.96±0.32^{a} (35)	9.74±0.33^{b} (33)	8.90±0.32^{a} (35)	9.65±0.36^{a} (27)	9.05±0.33^{a} (33)	10.4±0.32^{b} (34)
Cooked L^*	83.1±0.34^{a} (33)	76.4±0.34^{b} (34)	83.1±0.34^{a} (33)	78.4±0.33^{b} (35)	82.7±0.34^{a} (33)	75.5±0.34^{b} (34)
Cooked a^*	0.75±0.09^{a} (30)	1.98±0.09^{b} (33)	0.71±0.09^{a} (34)	1.93±0.09^{b} (34)	1.09±0.09^{a} (35)	1.97±0.09^{b} (34)
Cooked b^*	14.9±0.18^{a} (31)	15.8±0.19^{b} (30)	15.5±0.18^{a} (33)	16.2±0.18^{b} (33)	15.6±0.17^{a} (35)	16.0±0.18^{a} (32)
Firmness (N)	14.9±1.27^{a} (35)	33.6±1.29^{b} (34)	14.3±1.27^{a} (35)	37.5±1.27^{b} (35)	14.7±1.27^{a} (35)	46.2±1.27^{b} (35)
Work Penetration (N*sec)	47.0±3.58^{a} (34)	103±3.69^{b} (32)	46.5±3.69^{a} (32)	118±3.69^{b} (32)	50.0±3.53^{a} (35)	151±3.53^{b} (35)
Shear Force (N)	10.9±0.39^{a} (29)	12.6±0.37^{b} (33)	10.8±0.37^{a} (33)	12.3±0.36^{b} (35)	10.8±0.37^{a} (33)	15.8±0.37^{b} (33)
Shear Energy (N*mm)	15.1±0.56^{a} (31)	18.7±0.53^{b} (34)	14.5±0.53^{a} (35)	17.1±0.53^{b} (35)	14.3±0.57^{a} (30)	23.8±0.54^{b} (33)
Muscle tone (Hz)	25.1±0.21^{a} (33)	24.9±0.22^{a} (31)	26.1±0.21^{a} (34)	25.5±0.21^{a} (34)	25.8±0.21^{a} (34)	26.0±0.22^{a} (30)
Stiffness (N/m)	508±7.03^{a} (33)	575±6.92^{b} (34)	546±6.92^{a} (34)	602±6.92^{b} (34)	546±6.83^{a} (35)	612±6.92^{b} (34)
Elasticity	1.66±0.02^{a} (35)	1.45±0.02^{b} (31)	1.56±0.02^{a} (31)	1.32±0.02^{b} (35)	1.52±0.02^{a} (34)	1.33±0.02^{b} (34)
Relaxation (ms)	9.87±0.12^{a} (32)	9.13±0.11^{b} (34)	9.12±0.11^{a} (34)	8.65±0.11^{b} (33)	9.26±0.11^{a} (35)	8.50±0.12^{b} (32)
Creep	0.64±0.01^{a} (33)	0.60±0.01^{b} (34)	0.59±0.01^{a} (34)	0.58±0.01^{a} (35)	0.60±0.01^{a} (35)	0.57±0.01^{b} (31)

* Subscripts represent the comparative results of the breast condition within each test. Different letters indicate significant differences ($p<0.05$).

** The numbers in parentheses represent the sample size.

Table 3. Pearson correlations among parameters studied.

	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)[1]
(1) WB Score	0.48	-0.77	0.23	0.52	0.39	0.36	-0.74	0.68	0.28	0.77	0.77	0.51	0.59	-0.07*	0.56	-0.61	-0.38	-0.30
(2) pH		-0.44	0.08*	0.48	0.27	-0.06*	-0.48	0.38	0.07*	0.37	0.34	0.17	0.24	-0.29	0.13	-0.15	0.00*	0.05*
(3) Cooking Yield			-0.34	-0.45	-0.45	-0.40	0.87	-0.70	-0.24	-0.78	-0.76	-0.60	-0.69	0.02*	-0.56	0.58	0.40	0.34
(4) Water Loss				0.27	0.17	0.25	-0.33	0.26	0.10*	0.32	0.31	0.08*	0.14	-0.13*	0.16	-0.33	-0.09*	-0.09*
(5) Raw L^*					0.08*	0.40	-0.43	0.32	0.21	0.42	0.44	0.10*	0.14	-0.39	0.15	-0.41	0.04*	0.09*
(6) Raw a^*						0.20	-0.59	0.53	-0.04*	0.41	0.39	0.35	0.41	0.05*	0.28	-0.25	-0.23	-0.22
(7) Raw b^*							-0.38	0.30	0.47	0.33	0.35	0.15	0.17	-0.12*	0.26	-0.42	-0.15	-0.10*
(8) Cooked L^*								-0.81	-0.37	-0.76	-0.75	-0.60	-0.68	-0.02*	-0.59	0.56	0.42	0.35
(9) Cooked a^*									0.27	0.67	0.63	0.50	0.54	0.02*	0.53	-0.50	-0.37	-0.31
(10) Cooked b^*										0.25	0.29	0.08*	0.10*	-0.00*	0.25	-0.27	-0.15	-0.12*
(11) Firmness											0.99	0.61	0.67	0.11*	0.66	-0.67	-0.51	-0.45
(12) Work Penetration												0.59	0.65	0.10*	0.67	-0.70	-0.51	-0.44
(13) Shear Force													0.96	0.23	0.49	-0.30	-0.40	-0.36
(14) Shear Energy														0.24	0.52	-0.32	-0.44	-0.39
(15) Muscle tone															0.65	-0.12*	-0.79	-0.81
(16) Stiffness																-0.69	-0.97	-0.95
(17) Elasticity																	0.56	0.52
(18) Relaxation																		0.99

[1](19) Creep

* The correlation coefficients are not significantly different from zero ($p > 0.05$).

WB: Woody Breast