Journal of Veterinary Science & Technology

Harkati et al., J Vet Sci Technol 2018, 9:4
DOI: 10.4172/2157-7579.1000548

Research Article Open Access

Effect of Pre-slaughter Handlings on Broiler Chicken Welfare and Relationship with Meat Quality

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Received date: May 30, 2018; **Accepted date:** June 22, 2018; **Published date:** June 25, 2018

Abstract

The aim of the present work is to study pre-slaughter handlings effect on broiler chicken's well-being and its relationship with meat quality parameters. Animals hanged and electrocuted were significantly stressed and their meat has highest $pH_{30\ min}$ and cooking losses ($P<0.05$). Depths penetration in their transformed meat was lower than meat of animals bled directly. Significant variability was also showed in myofibrillar proteins. Alpha-actinin-2, Myosin heavy chain skeletal muscle adult, sarcoplasmic/endoplasmic reticulum calcium ATPase1, Tropomyosin alpha-1chain, Fructose bisphosphate aldolase C, Glyceraldehyde-3-phosphate dehydrogenase and L-lactate dehydrogenase A chain levels increased in stressed animals. These proteins were negatively correlated to Troponin C, skeletal muscle and Myosin regulatory light chain 2 which characterize unstressed animals. In conclusion, Stressed animals presented high glycaemia and serum cortisol. Their meat has high $pH_{30\ min}$, cooking losses, Glyceraldehyde-3-phosphate dehydrogenase and L-lactate dehydrogenase, a chain levels. Their transformed meats were less tender than unstressed animals.

Keywords: Pre-slaughter handlings; Poultry welfare; Meat quality; Myofibrillars proteins

Introduction

Stress accompanying animal production is both an indicator of welfare and factor that affects economic parameters [1]. Animals are at great risk of fear during the procedures that take them to new situations, such as pre-slaughter handling, which implies an important additional stress [2]. Pre-slaughter stress and how the animals physiologically respond to stress are factors that affect glycogen depletion in animals and meat quality parameters such as pH, color, cooking losses and tenderness [3].

In poultry sector, pre-slaughter stress is induced by interactions between humans and animals at transport and slaughter; stressors affect physiological statue of bird [4] and Loprz-Hellin et al. [5] reported that these changes could also alter protein content in muscle, reflecting many biochemical processes. Broilers in transit for a lengthy period had to cope with higher stress as demonstrated by their blood parameters and changes in their protein expression profile [6]. For that, pre-slaughter conditions are important in respect of quality and welfare of birds, so better understanding them will help modern poultry industry to obtain good quality [7].

From this perspective, the aim of our work was to study the effect of pre-slaughter handlings on broiler chicken's welfare and relationship with meat quality.

Materials and Methods

Thirty broilers (ISA 15 strain) were used, reared in accordance with rearing protocols of Algerian agriculture ministry, at East Avicol Group. At 54 days old, they were transported 2 h at accredited commercial slaughterhouse (Hammadi Krouma, Algeria) and rested 2 h. Three hallal slaughter modes were applied. Animals of group (B) were bled directly. Animals of group (SB) were hanged by paws and bled in this position. Animals of group (SEB), were hanged and through electro narcosis tray (low voltage) to be bled.

Serum collection and muscle sampling

Blood was collected in heparinized tubs at farm, after transport and at exsanguinations. Pectorals muscles were excided from carcasses after slaughter; they were transported with blood tubs at 4°C to laboratory. Muscles were refrigerated at 4°C until 24 h. For proteomic analyses, muscles were frozen in liquid nitrogen 24 h post mortem and kept at -25°C.

Physiological analyzes

Glycaemia was measured with colorimetric method following tow coupled enzymatic reactions using glucose-oxidase and peroxidase. Glucose concentration was read with spectrophotometer at 470 nm. Serum cortisol was determined by radioimmunoassay (RIA analyses) using commercial Kit (Elecsys 1010, ROCHE, Germany). In the tow analyzes we have follow equipment manufacturers instructions.

Technologic analyzes

Meat pH was measured by direct probe method using specific meat pH meter (Hanna-HI99163), values were taken at 30 min and 24 h post mortem. Cooking losses were determined [8]. Meat gel was prepared to study of meat suitability processing, Molette et al. [9] have describe gel preparation, gels were cut at cylinders (1, 2 cm diameter) and depth penetration (mm) was measured for cooked meat and

cylinder of meat gel using penetrometer (PETROTEST PNR 10 GERMANY) [10].

Proteomic analyzes

Proteomics provide useful things to improve meat tenderness or other meat qualities [11]. In our study we have established myofibrillar proteins profile and see relations with study parameters. For Extraction of muscle proteins, 0.5 gm muscle was cut to small pieces, agitated with 4 ml 10 Mm Tris-HCl (pH-7.6), 1 mM EDTA, 0.25 M sucrose and 25 µl inhibitors cocktail, homogenized in polytron and centrifuged (20 min, 4°C at 12000 rpm). Supernatant was filtered (0.45 µm). Pellet was washed in 4 ml of same buffer but without inhibitors cocktail, homogenized and centrifuged. Precipitate was dissolved in 4 ml 10 Mm Tris-HCl (pH-7.6), 7 M Urea, 2 M Thio-Urea and 10 mM DTT; homogenized and centrifuged. Supernatant constituted myofibrillar proteins extract. Protein concentrations were determined according to Bradford et al. [12] using Bio-Rad protein assay.

SDS-PAGE was used according to Laemmli method modified; 50 µl extract mixed with same volume simple buffer × 2 (0.5 M Tris-HCl, pH-6.8, 50% glycerol, 10% SDS, 0.2 M DTT and bromophenol bleu). Solution was heated for 4 min at 95°C in AccuBlock. 20 µg of proteins was loaded in each well and separated in continuous buffer system (0, 25 M Tris-HCl, 1.92 M glycine and 50% SDS) trough vertical gel 12% polyacrylamide. Electrophoresis was run 2 h at 50 mA (Hoefer SE 260 cuve). Gels were staining 1 h in 12% trichloro acetic acid, washed 10 min × 2 in Milli-Q Water and stained overnight in Coomassie blue (10% ortho-phosphoric acid, 10% ammonium sulfate, 12% Coomassie G-250 and 20% methanol). Gels were washed with Mill-Q water; images were obtained with FUJIFILM LAS-1000 and analyzed with Gel Analyzer Software.

For in-gel trypsin digestion, Protein bands found to be significatively different between groups samples were excised from gels, introduced into 0.5 mL Eppendorf tubes and washed 10 min with 50 mM ammonium bicarbonate × 3, pH-8.0. Gel pieces were dried for 10 min with 50 µL acetonitrile × 3.15 µL of 12.5 ng/µL trypsin, pH 8.0 and 15 µL of 50 mM ammonium bicarbonate were added. Tubes were incubated at 37°C overnight. Supernatant was transferred to new Eppendorf tube. Gel pieces were washed with 25 µL ACN/0.1% TFA, sonicated for 10 min and supernatants were combined. Solvent was evaporated using Savant SPD 121 P Speed Vac concentrator equipped with RVT400 refrigerated vapor trap and further re-dissolved in 40 µL 0.1% tri-fluoro acetic acid.

Peptide sequence identification by tandem mass spectrometry

25 µL peptide solution was injected using Surveyor LC system equipped with auto sampler and coupled to LCQ Advantage ion trap MS instrument through electrospray ion source. Peptides separation was carried out on Jupiter Proteo reversed-phase column (15.0 cm × 0.5 mm) Phenomenex, Torrance, California, U.S.A. Mobile phases consisted of solvent A contains 0.1% formic acid in bi-distilled water and solvent B contains 0.1% formic acid in acetonitrile. Separation conditions consisted of stepwise isocratic gradients including 15 min of 0% B, 5 min of 20% B, 10 min of 40% B and finally 10 min of 100% B at flow rate of 30 µL/min after which it was returned to its original gradient having 0% of solvent B. Operating parameters of ion trap detector were the following: electrospray ionization in positive mode, capillary temperature 250°C, collision energy normalized to 35%, spray voltage 4.0 kV and capillary voltage 15.0 V. First scan event was full MS detection for m/z values in range 400-2000. Second event was a dependent MS/MS scan of most intense ions having charges from +2 to +4, enabling dynamic exclusion after three scans for 10 min. Minimum ion intensity for triggering MS/MS scan was 105 intensity units. Data acquisition was done using Xcalibur v2.0 software. Peptide identification was done from information contained in generated MS/MS spectral data using in-house version of Mascot search engine v2.3 (www.matrixscience.com) against the Uniprot KB protein database (www.uniprot.org).

Statistical analysis

Results were analyzed using IBM SPSS statistics 24.0 software, measurements. ANOVA test was used for all results and significant differences between means were determined by Tukey's test at $P \leq 0.05$. Principal component analysis (CPA) was used to study correlations between study groups and protein bands using XLSTAT 2016 software.

Results and Discussion

Physiological parameters

Glucose at rest was 211.6 ± 8.39 mg/dl which is in standards interval (190-220 mg/dl) cited by Scane et al. [13]. After transport and waiting at slaughter house, values decreases ($p<0.05$) at 209.5 ± 12.98 mg/dl. Animals waiting for slaughter can be stressed by factors such as restraint, handling, pre-slaughter environment, adverse weather conditions, hunger and fatigue [14].

Parameters	B	SB	SEB	P-value	Significance
	N=10	N=10	N=10		
Physiological parameters					
Blood glucose (mg/dl)	180 ± 0.17	228 ± 0.30	243 ± 0.43	0	***
Blood cortisol (µg/ml)	0.16 ± 0.02	0.28 ± 0.10	0.38 ± 0.12	0.005	**
Technological parameters					
pH30 min	6.24 ± 0.07	6.52 ± 0.19	6.74 ± 0.16	0	***
pH24 h	5.86 ± 0.13	5.84 ± 0.13	5.95 ± 0.04	0.083	NS
Cooking loss (%)	18.24 ± 0.48	23.17 ± 0.42	26.37 ± 0.74	0	***
Depth penetration (mm)					
Cooked meat	1.27 ± 0.39	1.39 ± 0.49	0.81 ± 0.41	0.12	NS
Transformed meat	2.20 ± 0.24	2.27 ± 0.31	1.61 ± 0.36	0	***

Table 1: Physiological and technological parameters of animals and meat. (B, SB and SEB were groups of study animals).

After restrain-transport of broiler chickens blood glucose decreases ($p<0.001$) [15]. Zanetti et al. [6] indicate a greater tendency for stress responses in broilers in longer transit. After bleeding, blood glucose

increases (p>0.05) for animals in-group B but remains normal (Table 1). However for SB and SEB groups it increase significantly (p<0.05). Cortisol value at bleeding were also high (p>0.05) for SB and SEB groups successively relative to group B.

Hanging on slaughter line puts animals in agonizing position that results in significant increase in corticosterone levels [16]. Response to acute stress brings about cascade of physiological reactions, activating nervous sympathetic-adrenomedullary system which responds to short-term stress through catecholamine's production and hypothalamic-pituitary-adrenocortical system which involves an increase in plasma cortisol levels [17]. ANOVA analysis point out that animals slaughtered directly (group B) were unstressed and those of SB and SEB groups which were hanged and electrocuted were stressed (no significant differences (P<0.05) between SB and SEB).

Technological parameters

pH30 min for B group was higher (p< 0.05) than in SB and SEB. For chicken, increase of pH15 min post mortem is [18].pH after 24 h post-mortem is routinely used at slaughter houses to determine meat quality and track future processing decisions. In our study, birds of SEB have the highest (p>0.05) pH24 h. pH24 hof broilers [18]. Cooking losses for stressed animals (SEB and SB) were higher (p<0.05) than unstressed animals. However, Uzum et al. [19] found under stress that effect is not significant. Debut et al. [18] reported that electro narcosis has no significant effect on cooking losses in poultry. For cooked meat, difference of depth penetration was not significant (p>0.05). Electro narcosis has note an effect on tenderness [18]. However for transformed meat, the lowest (p<0.05) depth penetration was in SEB meat.

Proteomic parameters

Post mortem degradation of myofibrillar protein has been linked with post mortem tenderization of meat [20]. SDS PAGE separation of myofibrillar proteins have given profiles showed in Figure 1. 18-19 bands in general were found, qualitative and quantitative variability were illustrate in Table 2. The range of molecular weight detected was in agreement with profile presented by Adeymi et al. [21] for *Pectoralis major* in broiler chicken. For qualitative variability, we have absence of band M in group B. However, band O and Q were absent for SEB group. Protein containing in this bands were identified and presented in Table 2 with proteins of bands that differ significantly. Proteins identified in band M were Glyceraldehyde-3-phosphate dehydrogenase G3P and L-lactate dehydrogenase A chain (LDHA). For band Q: Troponin C, skeletal muscle (TNNC2) and Myosin regulatory light chain 2 (MLRS). Proteins of band O which have 23.4 kDa were not identified in our study. Concerning quantitative variability, among all bands detectable, only some bands were differently expressed to significant degree in group of samples divided by slaughter mode (P<0.05).

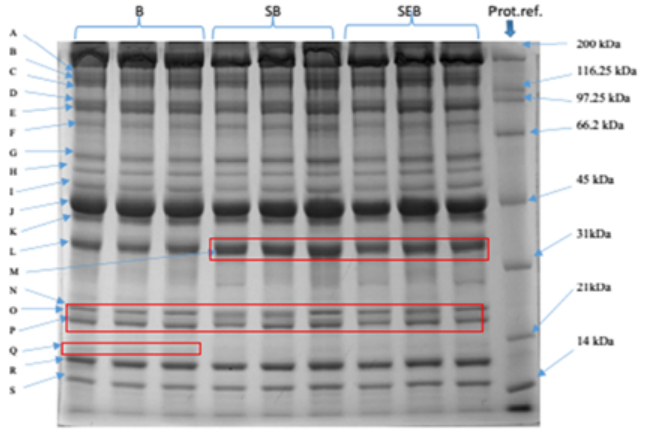

Figure 1: 12% SDS-PAGE of fractions obtained from myofibrillar extracts of the three studied groups (B, SB and SEB animals groups of study).

Band	P	Group	Identified protein	Name of protein	Molecular weight (Da)	Score
E	0.027	B	ACTN2-CHICK	Alpha-actinin-2	104209	227
			MYSS-CHICK	Myosin heavy chain, skeletal muscle, adult	223006	88
		SB	ACTN2-CHICK	Alpha-actinin-2	104209	127
			MYSS-CHICK	Myosin heavy chain, skeletal muscle, adult	223006	240
			AT2AI-CHICK	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	108953	231
		SEB	ACTN2-CHICK	Alpha-actinin-2	104209	395
			MYSS-CHICK	Myosin heavy chain, skeletal muscle, adult	223006	137
			AT2AI-CHICK	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	108953	55
K	0.030	B	TPM1-CHICK	Tropomyosin alpha-1chain	32746	190
			ALDOC-CHICK	Fructose biphosphate aldolase C (Fragment)	14429	102
		SB	TPM1-CHICK	Tropomyosin alpha-1chain	32746	164

			ALDOC-CHICK	Fructose biphosphate aldolase C (Fragment)	14429	89
		SEB	TPM1-CHICK	Tropomyosin alpha-1chain	32746	251
L	0.01	SEB	G3P-CHICK	Glyceraldehyde-3-phosphate dehydrogenase	35681	578
			LDHA-CHICK	L-Lactate dehydrogenase A chain	36491	159
M	0.000	SEB	G3P-CHICK	Glyceraldehyde-3-phosphate dehydrogenase	35681	170
			LDHA-CHICK	L-Lactate dehydrogenase A chain	36491	151
		SB	G3P-CHICK	Glyceraldehyde-3-phosphate dehydrogenase	35681	252
			LDHA-CHICK	L-Lactate dehydrogenase A chain	36491	89
Q	0.027	SEB	TNNC2-CHICK	Troponin c, Skeletal muscle	18364	239
			MLRS-CHICK	Myosin regulatory light chain 2	18827	190
		B	TNNC2-CHICK	Troponin c, Skeletal muscle	18364	159
			MLRS-CHICK	Myosin regulatory light chain 2	18827	53

Table 2: Identification of protein subject of variation MS/MS. (A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R and S protein bands).

Component principal analysis for all the bands shows 66.83% of variability. The first axe explain 49.86% of variability; bands M, R, L, S, B, H, K, D, G, P, F, E, and N were positively correlated and negatively correlated with band Q and O. The second axe explained 16.97% of variability, bands A, C and I were positively correlated. The Bi-plot of all bands and individual animals show that unstressed animals (B) were separated from SB and SEB and correlated with band Q especially (Figure 2); effect of slaughter mode on protein concentration is significant ($P<0.05$).

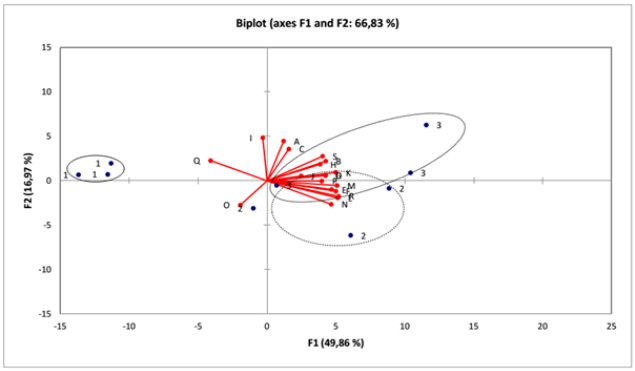

Figure 2: Bi-plot for distribution of animal study groups and correlation with myofibrillar proteins.

G3P is more abundant in stressed groups, it is a glycolytic pathway enzyme and levels were also higher in pigs with armed muscles because they were physically more active ante-mortem [16]. For stress induced by restraint transport, for broiler, G3P and L-lactate dehydrogenase involved in glycolysis were repressed [9]. Stressed broiler had a higher expression of some proteins related to energy metabolism as G3P, which impose higher energy to cope with harsh management conditions, hunger, temperature, shaking, noise and social disruption [21]. In addition, our results show absence of TNN2C in SEB group, which was probably been degraded.

Conclusion

Pre-slaughter handlings have significant effect on animal's wellbeing. $pH_{30\ min}$, cooking loss and depth penetration were also affected. Significant effect was determined on Alpha-actinin-2, Myosin heavy chain skeletal muscle adult, sarcoplasmic/endoplasmic reticulum calcium ATPase1, Tropomyosin alpha-1chain, Fructose bisphosphate aldolase C. Glyceraldehyde-3-phosphate dehydrogenase and L-lactate dehydrogenase A chain were correlated with stressed animals and negatively correlated to Troponin C, skeletal muscle and Myosin regulatory light chain 2. Stressed animals presented high values of glycaemia and serum cortisol, their meat present high pH30 min, cooking loss, Glyceraldehyde-3-phosphate dehydrogenase and L-lactate dehydrogenase A chain concentrations and their transformed meat were less tender.

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