CHANGES OF GLYCOLYTIC POTENTIAL, CITRATE SYNTHASE AND ANTI-OXIDANT ENZYMES IN NORMAL AND WOODEN BREAST *PECTORALIS MAJOR* MUSCLE

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I. INTRODUCTION

Chicken with fast growth rate and high breast yield have been selected to meet the growing poultry consumption demands [1]. However, the high growth rate has been linked to increased prevalence of wooden breast (WB) myopathy, exhibiting histological alterations including polyphasic myodegeneration and connective tissue accumulation [1], and metabolic disturbances [2]. Although the etiology of this disease remains unclear, WB pathogenesis is linked to oxidative stress [1]. In this study, glycolytic potential, and activities of citrate synthase and selected antioxidant enzymes were determined to explore the changes on the level of glycogen, mitochondria functionality and oxidative stress in WB muscles. In addition, total myofiber area, the area occupied by undamaged myofibers, was also measured to compared how the changes of glycolytic potential and citrate synthase correlate with total myofiber area in normal and WB muscles.

II. MATERIALS AND METHODS

Sample processing, WB evaluation and citrate synthase measurement were conducted according to Li *et al*. (2022). Total glucose was measured with a Roche diagnostic kit, lactic acid content with a Boehringer Mannheim diagnostic kit, and glycolytic potential was calculated according to Monin *et al*. (1985). Antioxidant enzyme activities were determined according to Carvalho *et al*. (2017). Total myofiber area was estimated applying histomorphometry. The data were analysed by means of IBM SPSS Statistics 26 program.

III. RESULTS AND DISCUSSION

In this study, glycolytic potential, activities of citrate synthase and antioxidant enzymes were used as indicators to respectively reflect glycogen level, mitochondrial functionality and oxidative stress level in muscles. Compared to normal muscles, WB muscles showed significantly lower level of total glucose, lactate, and glycolytic potential indicating a reduction of glycogen (Table 1). In agreement, Baldi *et al*. (2020) found a significant decrease of lactate, glucose content and glycolytic potential in WB muscle 24h post-mortem and they proposed that lower glycolytic potential in affected muscles indicates that reduced substrate (glycogen) supply may compromise glycolysis. Decreased citrate synthase activity indicated that mitochondrial functionality was impaired in WB muscle. However, no significant difference between normal and WB muscle was observed in glycolytic potential and citrate synthase activity when normalized to myofiber area (Table 1). This result demonstrates that these two indicators decrease at similar rate as the muscle fibers, and the lower values in WB muscles thus derives from the smaller volume of functioning fibers, not the properties of the fibers.

Moreover, WB muscles exhibited significantly higher activities of catalase, superoxide dismutase and glutathione peroxidase. Similar results also found by Pan *et al*. (2021). These results imply a higher level of oxidative stress in affected birds. Oxidative stress has been widely considered as an important factor related to WB development and may contribute to the imbalance of pro-oxidants and endogenous antioxidants, which will lead to the accumulation of reactive oxygen species (ROS) and overproduction of free radicals induced by hypoxia [1]. Increased activity of antioxidant enzymes could be a response of the muscle to reduce the formation of excessive free radicals aiming to counteract oxidative stress. In addition, oxidative stress may also promote mitochondrial damage leading to muscle fiber dysfunction and changes in metabolism, including carbohydrate metabolism [2].

Table 1. Glucose and lactate concentrations, glycolytic potential, and citrate synthase and antioxidant enzyme activities in the normal and wooden breast *pectoralis major* muscle.

	n	Glucose µmol/g	Lactate µmol/g	Gly.pot. µmol/g	CS µmol/ (ml*min)	CAT U/ g protein	SOD U/ g protein	GPx U/ g protein	Gly.pot/ TMA µmol/g/%	CS/TMA µmol/ (ml*min)/%
Normal	12	6.1^{a}	105^{a}	117^{a}	2.1^{a}	48^{a}	30^{a}	0.43^{a}	1.37^{a}	0.024^{a}
WB	23	2.0^{b}	93^{b}	98^{b}	1.7^{b}	77^{b}	41^{b}	0.69^{b}	1.55^{a}	0.026^{a}
SEM		0.46	2.9	3.2	0.058	3.3	1.4	0.041	0.069	0.001
Effects		**	*	**	**	**	**	**	ns	ns

Glycolytic potential and citrate synthase activity are also expressed divided by total myofiber area. SEM: standard error of mean. Within each indicator, superscripts without the same letter differ ($P < 0.05$). WB: wooden breast; Gly.pot.: glycolytic potential; CS: citrate synthase; CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; TMA: total myofiber area. n: the number of breast fillets. One unit (U) of CAT activity is the amount of extract needed to decompose l µmol of H_2O_2 per min. One unit of SOD is the amount of extract needed to inhibit the pyrogallol autoxidation by 50%. GPx activity is the amount of extract needed to oxidise 1 µmol of NADPH per min at 25 °C. ** $P < 0.01$; * $P < 0.05$; ns: not significant.

IV. CONCLUSION

Both glycolytic potential and citrate synthase activity decreased in WB muscle, while activity of antioxidant enzymes increased, suggesting that WB exhibits higher oxidative stress accompanied by mitochondrial dysfunction and reduced level of glycogen. The glycolytic potential and citrate synthase values, normalized to the total fiber area, do not differ in normal and WB muscles, suggesting that energy metabolism of unaffected fibers are similar in both muscles, but their relative volumes differ.

REFERENCES

1. Petracci, M., Soglia, F., Madruga, M., Carvalho, L., Ida, E., & Estévez, M. (2019). Wooden-breast, white striping, and spaghetti meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. Comprehensive Reviews in Food Science and Food Safety 18(2): 565-583.
2. Abasht, B., Mutryn, M. F., Michalek, R. D., & Lee, W. R. (2016). Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. PLoS One 11(4): e0153750.
3. Li, B., Dong, X., Puolanne, E., & Ertbjerg, P. (2022). Effect of wooden breast degree on lipid and protein oxidation and citrate synthase activity of chicken pectoralis major muscle. LWT–Food Science and Technology 154: 112884.
4. Monin, G., & Sellier, P. (1985). Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: The case of the Hampshire breed. Meat Science 13(1): 49-63.
5. Carvalho, R. H., Ida, E. I., Madruga, M. S., Martínez, S. L., Shimokomaki, M., & Estévez, M. (2017). Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry meat. Food Chemistry 215: 129-137.
6. Baldi, G., Yen, C.-N., Daughtry, M. R., Bodmer, J., Bowker, B. C., Zhuang, H., Petracci, M., & Gerrard, D. E. (2020). Exploring the factors contributing to the high ultimate ph of broiler pectoralis major muscles affected by wooden breast condition. Frontiers in physiology 343.
7. Pan, X., Zhang, L., Xing, T., Li, J., & Gao, F. (2021). The impaired redox status and activated nuclear factor-erythroid 2-related factor 2/antioxidant response element pathway in wooden breast myopathy in broiler chickens. Animal Bioscience 34(4): 652-661.