Relationship between ultimate pH and stress-related blood variables in cattle

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Abstract

DFD(Dark，Firm and Dry)beef was also called dark cutting beef, which exhibited a dark, purplish red to almost black lean color and a dry, often-sticky lean surface. Due to high ultimate pH, DFD beef had a greater ability to support microbial growth and a reduced shelf life. Pre-slaughter stress was closely related with meat quality. Under stressful situations, the physiological response may influence the rate and extent of muscle metabolism, resulting in inferior beef quality. The objective of this study was to investigate the relationship between ultimate pH and some stress-related blood variables in cattle. Compared to cattle with normal ultimate pH (pH_u<5.8), cattle with higher ultimate pH (pH_u>6.1) showed higher plasma cortisol, glucose, lactate, creatine kinase and lactate dehydrogenase levels at exsanguination. But the concentration of haptoglobin and serum amyloid A protein showed no significant differences among three pH groups. These results imply that measuring plasma cortisol, glucose, lactate, creatine kinase, or lactate dehydrogenase levels at exsanguination may be available to identify the dark-cutting beef.

Key Words- ultimate pH, blood variables, dark-cutting beef

I. INTRODUCTION

Cattle before slaughter encounter many stressors inevitably, including transportation, novelty, fasting, noise and so on [1]. Pre-slaughter stress may result in the development of dark-cutting (DC) beef because of depletion of antemortem muscle glycogen stores and shortage of postmortem lactic acid accumulation. When an animal is stressed under stressful circumstance, there is a rapid release of catecholamines from the adrenal medulla which result in glycogen depletion causing high ultimate pH and dark-cutting beef [2]. Beef from carcasses with high ultimate pH values produces undesirable surface, shorter shelf life, bland flavour and variable tenderness [3]. So how to avoid stress prior to slaughter is significant not only for improving animal welfare but also for keeping beef quality.

Some studies have demonstrated that stressors such as transport and lairage may affect some stress-related blood variables, such as cortisol, glucose, lactate, creatine kinase, lactate dehydrogenase [4-7]. And almost every procedure before slaughter may impact the blood biochemical indicators in cattle to some degree.

The objective of this study, therefore, was to investigate the relationship between ultimate pH and some stress-related blood variables at exsanguination. We hypothesize that cattle with higher ultimate pH will have higher levels of plasma cortisol, glucose, lactate, creatine kinase, and lactate dehydrogenase.

II. MATERIAL AND METHODS

A. Animals and slaughter procedure

A total of 20 Friesian bulls (pure breed) were selected on the slaughter line from a commercial feedlot (Sishui Xinlv Food Co., Ltd., China). All the cattle were transported from commercial farm to slaughter house within 4h and spent approximate 14h in the lairage before stunning. All animals were restrained in the stunning box, after captive bolt stunning, the animals slid out of the stunning box and were elevated by one hind-leg. Suspended cattle were exsanguinated by throat cut. Then the carcasses were split into the left and right sides and cooled in the chilling room (4°C) for 48 h postmortem.

B. Blood samples and measurements

Blood samples were collected from each cattle into two types of evacuated tubes (Vacutainer, BD) within 30s during exsanguination after stunning and sticking. The first tube was treated with potassium oxalate/sodium fluoride, and the second tube was treated with heparin. Plasma lactate and glucose concentrations were analyzed from the first tube. Plasma cortisol, haptoglobin (Hp), creatine kinase(CK), lactate dehydrogenase (LDH), serum amyloid A (SAA) were estimated from blood collected in heparin tubes. Blood samples were centrifuged at 3000rpm for 10 min and the

plasma stored at -80°C until it was analyzed.

C. Carcass ultimate pH measurement

Carcass ultimate pH was measured at 24h after slaughter using a portable pH meter (SenvenGo, Mettler-Toledo, Switzerland) which was calibrated in buffers with pH 4.00 and 7.00. The probes were inserted into the center (about 3 cm) of the muscles between the 12th and 13th ribs. Each carcass was measured three times.

D. Statistical analysis

For the statistical analysis the PASW Statistics 18 software was used. All variables were presented as means with standard deviations. The relationship between ultimate pH and stress-related blood variables (glucose, lactate, creatine kinase, lactate dehydrogenase, cortisol, serum amyloid A and haptoglobin) were evaluated using the analysis of variance.

III. RESULTS AND DISCUSSION

Table 1

Means and standard deviations (S.D.) of blood variables in groups categorized by different ultimate pH

Variable	pH<5.8(n=6)	pH=5.8-6.1(n=8)	pH>6.1(n=6)
Cortisol （nmol/L）	48.81±8.05^{c}	90.01±9.44^{b}	170.22±22.58^{a}
Glucose （mmol/L）	5.51±0.65^{b}	5.79±0.53^{ab}	6.48±0.73^{a}
Lactate （mmol/L）	6.26±0.32^{b}	6.41±0.32^{b}	7.37±0.29^{a}
CK（U/L）	542.33±162.35^{c}	1243.50±191.03^{b}	2830.83±349.44^{a}
LDH（U/L）	770.17±69.93^{c}	1053.63±117.27^{b}	1305.00±119.17^{a}
Hp（mg/L）	403.64±60.39	404.20±67.86	429.17±97.76
SAA（g/L）	156.95±34.68	145.79±30.62	156.04±39.96

Different letters in the same row represent statistical differences (P<0.05).

Blood samples were classified into three groups based on the carcass ultimate pH values. All the experimental blood parameters are presented in Table 1.

In this study, animals with high pH had higher plasma cortisol concentration (170.22±22.58nmol/L) as compared to those with medium pH (90.01±9.44nmol/L) and normal pH (48.81±8.05nmol/L), which is consistent with Chulayo and O'Loughlin who has demonstrated that stressors such as transport, lairage, weaning impact the concentration of cortisol [8-9]. The main cause of high ultimate pH in cattle is due to pre-slaughter stress which depletes muscle glycogen reserves prior to slaughter and keeps a low level of lactate after death. Cortisol is generally considered as the primary biochemical indicator used to measure pre-slaughter stress [10]. The hypothalamic–pituitary–adrenocortical axis and the sympathetic nervous system are the main stress-responsive neuroendocrine systems. And they are easily stimulated by pre-slaughter stress. Activating the hypothalamic–pituitary–adrenocortical axis results in releasing cortisol into the blood [11], which explained the significant increase in cortisol level in high pH group.

Glucose level of high pH group were higher (6.48±0.73mmol/L) than those of normal pH group (5.51±0.65mmol/L) while the same trend was observed for lactate (7.37±0.29mmol/L and 6.26±0.32mmol/L). Previous studies have shown lameness significantly increases the concentration of blood glucose in cows, and cattle undergoing electric goads have higher plasma lactate at slaughter, indicating that changes in blood glucose and lactate levels may relate with pre-slaughter-stress [12-13]. As a consequence of variable lengths of transport, the reduction of ATP before animal slaughter results in the depletion of glycogen which inversely increases plasma glucose production [14]. Lactate concentration quickly elevates by physical behavioral reactions to stress. The adrenal medulla releases catecholamine when pre-slaughter stress activates the sympathoadrenal system. Thus, glycogen from the liver rapidly breakdown and the levels of blood glucose and lactate increase [15].

The high pH group had 2830.83±349.44U/L of CK activity and 1305.00±119.17U/L of LDH activity, which is higher than other two pH groups. The medium and low pH groups were also significantly different: CK activity (1243.50±191.03 vs. 542.33±162.35U/L, respectively) and LDH activity (1053.63±117.27 vs. 770.17±69.93U/L, respectively). Creatine kinase is a kind of specific enzyme in myocyte. When muscle is damaged, CK is released into the blood rapidly. Therefore, the activity of CK is always regarded as an indicator of stress response related to fatigue and muscle metabolism. Werner et al. (2013) find that CK activity increases after transport and suggest that pre-slaughter stress may elevate the activity of CK in cattle [16]. Lactate dehydrogenase is very similar to creatine kinase, which is released into blood after muscle damage and vigorous exercise. The result of present study is in agreement with Mohan Raj who reported that CK and LDH was positively correlated with pHu [17].

Acute phase proteins (APPs), such as haptoglobin and serum amyloid A protein, are a group of blood proteins that may be easily changed in concentration when animals were in stressful conditions [18]. So APP

response is considered as a very important indicator of pre-slaughter stress. Qiu reported maximum concentration of acute phase proteins is typically reached within 24–48 h after the initiation of the stimulus [19]. And many studies have demonstrated that the highest values of acute phase proteins were found 24 to 48h after transport [20-21]. But in this study, the concentration of haptoglobin and serum amyloid A protein showed no significance among three pH groups, partially due to the cattle in this experiment that were slaughtered within 14 hours after transport.

IV. CONCLUSION

The level of plasma cortisol, creatine kinase and lactate dehydrogenase were significantly different among three pH groups, and the concentration of plasma glucose and lactate in normal pH group were significantly lower than that in high pH group. But the concentration of haptoglobin and serum amyloid A protein showed no significance among three pH groups. These results imply that measuring plasma cortisol, glucose, lactate, creatine kinase, or lactate dehydrogenase levels at exsanguination may be available to identify the dark-cutting beef.

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