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Effect of Stress on Haemato-biochemical Parameters Alteration during Slaughter in Pigs

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A B S T R A C T

The objective of the study was to assess the changes acquired in haematological and biochemical parameters attributed to stress during slaughter in pigs. The study was conducted in twenty pigs. The blood sample was collected under aseptic condition from twenty pigs before slaughter at instructional pig farm and during slaughter from abattoir. On statistical analyses (mean was compared with t-test), it was observed that the haemoglobin, total erythrocyte count decreased and packed cell volume increased significantly ($P<0.05$). Biochemical parameters; albumin, Aspartate Transaminase and creatinine kinase significantly increased ($p<0.05$), while globulin, Blood Urea Nitrogen and creatinine decreased significantly ($p<0.05$). Significant increase ($p<0.01$) in of concentration cholesterol and total protein were recorded. Concentration of sodium and chloride decreased significantly ($p<0.01$) and potassium was increased significantly ($p<0.01$) due to slaughter. The study concluded that animals after slaughter can show metabolic alteration that leads to hyperglycaemia, increased lactate and descent of pH.

Introduction

Blood circulating in the body, carrying substances to and fro, is the first by-product obtained after slaughtering an animal. The meat quality has a direct association with pre slaughter handling and even animals treated under top animal-welfare conditions may have their meat quality compromised if handling is not appropriately performed**.** Handling of animal's pre and post slaughter affecting quality of meat can be assessed by studying the haemato-biochemical parameters (Louise *et al.*, 2014). It is also the primary source of animal protein. Some cultures in India and the world, consume blood as food, often in combination with meat (Davidson, 2006).

Haematological parameters are good indicators of the physiological status of animals (Adenkola and Durotoye, 2004). It is also an excellent medium for the measurement of potential biomakers, because its collection is relatively noninvasive and it encompasses an enormous range of

physiological process in the body at any given time (Anderson and Anderson, 2002; Ginsburg and Haga, 2006).

Information from biochemical profile is used to determine the pig health condition. Transport is stressful for pig, decrease animal welfare and meat quality (Mota-Rojas *et al.*, 2006; Becerril-Herrera *et al.*, 2007) evaluate several blood variables and their relations to stress. It is reported that animal transportation and lairage causes acute stress and it affects the hematological and biochemical parameters ultimately has a negative effect on meat quality (Averos *et al.*, 2007).

Thus, the present work was aimed to assess the alteration in haemato-biochemical parameters based on handling of pigs during slaughter. There are large number of previous reports were present on the haemato-biochemical profile alteration in blood for meat animals but in pigs, very few studies have done so far in respect to pre slaughter transport, lairage stress on blood haemato-biochemical parameters. Hence the present study was to evaluate haemato-biochemical changes of pig blood before and after slaughtering.

Materials and Methods

The study was carried out by aseptic collection of blood sample from twenty pigs, at pre-slaughter stage at instructional pig farm and from the same pigs during slaughter from local abattoir in heparinized and non-heparinized vials.

Blood was collected by jugular vein puncture and at exsanguinations during slaughter (Salajpal *et al.*, 2005). The non-heparinized blood samples were centrifuged at 2500 rpm for 10 min. The obtained serum was refrigerated at 2^0C for further biochemical analysis.

Haematological analysis

Blood samples were analyzed for packed cell volume (PCV) using microhaematocrit method, total erythrocyte count (TEC) using haemocytometer method, haemoglobin (Hb) concentration, blood indices (MCV, MCHC, MCH) as described by Schalm *et al.*, (1975). The pH was measured in blood before and after slaughter, using a Testo 205 pH-meter.

Biochemical Analysis

Total protein was estimated by the biuret reaction (Peters *et al.*, 1982), serum albumin by bromocresol green method, globulin by calculating the difference between total protein and albumin, glucose was estimated by Folin-Wu method, Creatinine was determined by the Jaffe reaction method (Seaton and Ali, 1984) and BUN (Blood urea nitrogen) by diacetyl mono-oxime methods described by Harold, 1988. Serum lactate estimated through the Chemiluminescence and Kinetic enzymatic techniques; serum calcium and cholesterol were determined using the procedure described by Kaneko *et al.*, (2008). Sodium and potassium were estimated by Flame photometery (Hawks *et al.*, 1954).

Serum enzymes Phosphorus and chloride (Cl^-) Aspartate Transaminase (AST) and creatinine kinase (CK) were estimated by commercial kit, manufactured by ERBA Company Limited, with Semi-autoanalyzer. The standardized protocol provided with the ERBA kit was followed for estimation.

Statistical analysis

Statistical analysis was done with statistical package for social sciences (SPSS) statistical software version 11.0 (Grade *et al.*, 2010). Comparison of different parameters in before and after slaughtered blood was done by t-test.

Results and Discussion

Haematol-biochemical values before and after slaughter are shown in Table No. 1. Pre-slaughtered calculated values of Haemoglobin concentration, PCV, TEC, MCV, MCH, MCHC were 10.31± 0.32, 30.86± 1.54, 6.54± 0.81, 47.18± 0.21, 15.76± 0.35, 33.40± 0.15 respectively; whereas in Post slaughter 9.64± 0.24, 31.27± 0.45, 5.40± 0.64, 57.90± 0.01, 17.85± 0.15, 30.82± 0.30 respectively. In the present study Hemoglobin, total erythrocyte count and MCHC were decreased significantly ($p<0.05$). It was observed the values of packed cell volume, MCH, MCV increased significantly ($p<0.05$).

Assessment of biochemical parameters in Table No. 1 revealed that total protein and albumin concentration increased in slaughtered blood from 2.41±0.35 to 3.61±0.14 and 7.45±0.42 to 8.91±0.30 respectively while globulin value before and after slaughter 5.34±0.07 and 5.03 ±0.16; decreased significantly ($p<0.05$). Aspartate Transaminase and Creatinine Kinase (CK) value in Pre-slaughter blood was 27.50±0.89 and 462.59±14.82; for post slaughter 32.54±0.64 and 594.65±10.38 value increased significantly ($p<0.05$). There was significantly ($p<0.01$) increased value of glucose from 85.25±1.05 to 113.50±1.89 and cholesterol 52.08 ± 1.58 to 102.12 ± 4.32 were observed. Blood lactate (5.10±0.35 to 21.67±0.14) was also increased significantly ($p<0.05$) after slaughter. Pre-slaughter and post slaughter values of BUN were 22.46 ± 0.36 and 16.16 ± 0.86 respectively. Pre-slaughter value (1.42 ± 0.36) of creatinine was significantly higher ($p<0.05$) than post-slaughter values (1.05± 0.41).

Blood calcium (Ca) and phosphorous (P) concentrations decreased significantly ($p<0.05$) from 8.94 ± 0.27 to 4.96± 0.35 and 2.54±0.17 to1.94 ± 0.19 after slaughter. Before and after slaughter sodium concentration was 143.6±1.66 and 84.6±11.66 respectively; chloride concentration was 98.34±8.74 and 67.85±5.59; potassium concentration was 2.42±0.12 and 4.56±0.06. Blood Sodium and Chloride concentrations decreased significantly ($p<0.01$) whereas blood Potassium concentration was increased significantly ($p<0.01$) after slaughter.

Total protein and albumin concentration increased significantly due to slaughter, while globulin decreased significantly. This observation is supported by the report of Rojas *et al.*, (2009) for pig. The significant alternation in albumin and globulin is attributed to pre-slaughter and stunning stress. Hemoglobin and TEC level decreased in after slaughtered due to blood loss due to slaughter, albumin concentration might have increased, due to increase in packed cell volume, owing to dehydration or splenic contraction, induced by sympathetic nerve activity or circulating catecholamines (Tadich *et al.*, 2005). The explanation behind the decrease of globulin might be the fact that stunning stress might have caused reduction of immunoglobins by immuno-supression (Lee *et al.*, 2000). Hence, the increase of albumin compensating globulin decrease prevented the alternation of total protein.

Aspartate Transaminase increased in post slaughtered blood. Werner *et al.*, (2010) reported significant increased Aspartate Transaminase activity within 40 min postmortem in Duroc-Pietrain crossbreed pig. After 12 h, the activity of the enzyme decreased to the amount of the pre-slaughter samples. They concluded that Aspartate Transaminase influence the muscle-to-meat transition process after slaughter of the animals without an impact on the muscle quality.

Table.1 Haemato-biochemical parameters of pre and post slaughter of pig blood

S. No.	Parameters	Pre-Slaughter	Post Slaughter
1.	Albumin (g/dl)	2.41^{a}±0.35	3.61^{b}±0.14
2.	Blood urea nitrogen (mg/dl)	22.46^{a} ± 0.36	16.16^{b} ± 0.86
3.	Calcium (mg/dl)	8.94^{a} ± 0.27	4.96^{b} ± 0.35
4.	Cholesterol (mg/dl)	52.08^{c} ± 1.58	102.12^{d} ± 4.32
5.	Chloride (mmol/L)	98.34^{c}±8.74	67.85^{d}±5.59
6.	Creatinine (mg/dl)	1.42^{a} ± 0.36	1.05^{b}± 0.41
7.	Globulin (g/dl)	5.34^{a}±0.07	5.03^{b}±0.16
8.	Glucose (mg/dl)	85.25^{c}±1.05	113.50^{d}±1.89
9.	Lactate (mmol/l)	5.10^{a}±0.35	21.67^{b}±0.14
10.	Potassium (mmol/L)	2.42^{c} ± 0.12	4.56^{d} ± 0.06
11.	Phosphorus (mmol/L)	2.54^{a}± 0.17	1.94^{b} ± 0.19
12.	Sodium (mmol/L)	143.6^{c}± 1.66	84.6^{d}± 11.66
13.	Total bilirubin (mg/dl)	0.14^{a}± 0.07	0.28^{b} ± 0.09
14.	Total protein (g/dl)	7.45^{c}±0.42	8.91^{d}±0.30
15.	AST (U/L)	27.50^{a}±0.89	32.54^{b}±0.64
16.	Creatine kinase (U/L)	462.59^{a}±14.82	594.65^{b}±10.38
17.	Blood pH	7.34^{a}± 0.01	7.03^{b}± 0.01
18.	Haemoglobin	10.31^{a}± 0.32	9.64^{b}± 0.24
19.	Packed Cell Volume (PCV)	30.86^{a}± 1.54	31.27^{b}± 0.45
20.	TEC($\times 10^{6}/mm^{3}$)	6.54^{a}± 0.81	5.40^{b}± 0.64
21.	MCV (fl)	47.18^{a}± 0.21	57.90^{b}± 0.01
22.	MCH (pg)	15.76^{a}± 0.35	17.85^{b}± 0.15
23.	MCHC (g/dL)	33.40^{a}± 0.15	30.82^{b}± 0.30

Note: Mean with superscripts (a, b) in a row differ significantly ($p<0.05$). Mean with superscripts (c, d) in a row differ significantly ($p<0.01$).

Creatinine Kinase (CK) increased in blood concentration which also supported the result of Smiecinska *et al.,* (2011). Serum CK activity was higher in blood samples collected during carcass bleeding than in samples collected before, pointing to a strong stress response of animals to pre-slaughter treatment. They suggested that rest before slaughter alleviated stress, induced by pre-slaughter handling operations.

There was significantly increased value of glucose and cholesterol. *Guha et al.,* (2012) reported that slaughter caused hyperglycemia in buffaloes. Averos *et al.,* (2007) also reported significant increase of blood glucose in post slaughter blood in pigs. Cortisol produces more glucose by acting on the liver, increasing the synthesis of some enzymes which promote gluconeogenesis, in order to provide the body with instant energy (Werner and Gallo, 2008). The high sugar levels, however, often are not used up by the body and eventually are converted to fatty acids and cholesterol (Coleman *et al.,* 1998). Lynch *et al.,* (1964) attributed the increase of blood glucose level to rapid glycogenolysis in the liver after death.

Increased level of cholesterol and Lactate observed in post slaughtered pigs. These results were similar to those found Warriss *et*

al., (1994), in which the pigs subjected to high stress had higher levels of cortisol and lactate. In the pre-slaughter handling, animals get severely stressed, a condition that leads to increased levels of cortisol and lactate in their bloodstream, and may have as consequence a decrease in the meat quality (D'Eath *et al.*, 2010). Present study decreased value of BUN and creatinine, same was observed in the study of Marai *et al.,* (2006). They attributed it to stress, which might be due to heat, psychotic or stunning. It was observed that blood calcium (Ca) and phosphorous (P) concentrations decreased after slaughter. Significant hypocalcemia was also reported by Mandal *et al.,* (2013) in slaughtered goat, this might be due utilization of Ca ions by calpain proteolytic system Calcium is also utilized to maintain heart-beat and blood clotting mechanism (Kaneko *et al.,* 2008). Phosphorous required to carry out vital body functions. The significant decrease of Phosphorus is attributed to their utilization during body exposure to stress bearing factors during slaughter (Wojcik *et al.*, 2009).

Slaughter also affected the electrolyte profile, which plays an important role in homeostasis, acid-base balance, osmotic pressure, neural transmission, etc. Earlier, Wojcik *et al.*, (2009) reported similar observation for broiler chicken. Schaefer *et al.,* (1997) reported alternation in electrolyte profile in post slaughter blood of pigs, which they attributed to transport stress. They proved it, by supplementing electrolytes in drinking water of pigs, during and after transport. Death will cause the potassium (K) to be released from tissue or from liver into the blood. During stress, epinephrine might play a role in the release of K from brain cell in the blood. To maintain the electrical neutrality, sodium (Na) will move from the blood into the cells to carry out vital function at death. Chloride (Cl^-) moves with the electrical gradient along with Na (Lynch *et al.,* 1964).

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