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Research Article

Indigenous transport methods: Hormonal responses and physicochemical properties of lamb meat

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Abstract

The purpose of this study was to determine the effects of indigenous transport methods on hormonal responses and physico-chemical meat quality in lambs. The lambs (n=10) were exposed to different indigenous transport methods: NT (non-transport group), DT (3 h direct transport), WT (30-minute walking before 3 h direct transport), and WTW_{HJ} (30-minute walking before 3h transport and afterwards 30-minute walking in human movement). Blood samples were collected via jugular venipuncture from TS and NT lambs. Indigenous transport methods significantly decreased the functions of T_3, T_4 and TSH hormones compared to the non-transport group. On opposite, cortisol values were triggered by straight-cut journey and found decreased with the rest. Lambs upon exposure to indigenous transport methods showed elevated ultimate pH values than non-transport lambs. Both drip and cooking loss was significantly lower in indigenous transport protocols as compared to the control. The opposite trend was observed in case of water-holding capacity parameter. CIE L*, b* and a* values were significantly altered by indigenous transport methods. In conclusion, the present study indicated that indigenous transport methods may able to disturb the hormonal functions and also to produce poor grade lamb.

Introduction

Small ruminant takes up an important place as livestock resource in the agro-based economy of the country having greater significance, particularly, in subsistence agricultural operation. Sheep play a crucial role in the economy of smallholders in Bangladesh (Hashem et al., 2020; Hossain et al., 2021a and 2021b; Rana et al., 2014; Sun et al., 2020).Total number of sheep in Bangladesh is about 3.68 million where as 52.4% of the total them are kept by landless and small farmers and the rest 47.67% is kept by the medium and large farmers (BBS, 2011; DLS, 2020). They provide a substantial amount of meat to the people of Bangladesh. Sheep is reared by rural peoples under semi-intensive or extensive production system (Huq, 1988). Although the total cultivated land area has not changed since 1971, there is a sharp increase of human population and their living standard, and also the demand for animal-sourced protein. It is recently reported by DLS (2020) that Bangladesh is self-reliant for the production and supply of animal origin food. Now questions are arising for the origin and quality of that produced meats. In goats, there is evidence that transport stressed-goat produces very poor quality meat compared to non-transported goats (Salahuddin et al., 2019). Different pathways may probably be involved with the production of such low quality meat (Azad et al., 2021; Briggs, 2020). The one is compromised health and welfare issues that are incurred by transportation stress. During transportation, livestock experience to a variety of potential stressors, such as adverse climatic conditions (hot-humid summer, rainy or cold seasons, and humidity), loading and unloading episode, feed withdrawal, dehydration, and behavioral and movement limitations, and muscular damage (Collins et al., 2018; Padalino et al., 2018). The other one is the intensity of stress which could has potential to meat quality. Defects in meat quality arethe common phenomenon under either 'short-term' or 'long-term' treatments of transportation methods (Greaser, 2001; Gregory 2007). The pale, soft and exudative (PSE) is more associated with short-term stress. Briefly, the fast glycolysis (rapid drops in pH) and high pre-rigor temperature (37°C) creates an unfavorable condition within muscle that lead to denature and oxidize protein, resulting in formation of pale color. Simultaneously, water-holding capacity (WHC) is reduced as denatured proteins cannot hold, or bind muscular water, results a toughened meat. Contrarily, dark, firm, and dry (DFD) meat is associated with long-term stress prior to harvest animals. DFD meat is characterized as having an abnormally dark color, a firm texture, and an increased WHC associated with a dry, sticky surface. DFD meat originates from animals with deficient muscle glycogen levels at slaughter so that the conversion of muscle to meat through glycolysis (rigor) is impaired. Both PSE and DFD induces significant losses to the meat industry by altering color and tenderness as consumers consider tenderness and steak color to be two important attributes associated with meat quality. In the present meat industry of Bangladesh, transport stress is being considered to major problem. Because most of this transport is by roads especially village roads and the vehicles used for transportation are open trucks.

Moreover, the followings are absolutely unavailable including space requirements, journey duration and distance, rest stops, driver qualifications, environmental conditions, novel environments, lairage, road type while livestock are transported from their source to slaughter point. On opposite, in developed countries, the aforementioned prerequisites are strictly followed during transporting live animals from farms to slaughter yard. A significant research gap is seen between our and developed countries practices which needs to be address in terms of hormonal responses and physiochemical traits in lamb. Such information is highly demanding in Bangladesh. Therefore, the objective of the present experiment was to determine the hormonal regulation and meat quality of lambs exposed to indigenous transport methods.

Materials and Methods

Animals and experimental design

About 44 lamb with similar BW (±13.85) were purchased from near markets. Sheep, Goat and Horse farm under Department of Animal Science was used as baseline station for conducting transportation stress. The transportation stress protocol was: NT (non-transport group), DT (direct 3 h transport), WT (30-minute walking before 3 h direct transport), and WTW_{HJ} (30-minute walking before 3 h direct transport afterwards again 30-minute walking in human movement). Each group has 11 lambs.

Collection of blood sample and separation of serum

Jugular vein was selected for blood collection from all experimental animals. The site of collection was made sterile by using ethyl alcohol to prevent the risk of phlebitis. Immediately after collection into syringe, blood was transferred to sterile test tube containing anticoagulant (4% Sodium Citrate solution) at a ratio of 1:10. Because of time constraint, this blood was then carried out to Physiology Laboratory in an ice carrier and preserved under refrigeration temperature. The sample was then examined for various hematological parameters. The watery portion of blood after coagulation, a fluid found when clotted blood is left standing long enough for the clot to shrink is known as serum. The procedure of serum collection is as follows a. Blood was collected with sterilized syringe and needle without anticoagulated. b. The blood was kept in screw capped test tube and sometimes in small conical flask or disposable syringe itself. c. The test tube was kept at room temperature for 1 to 2 hours. d. Blood clots then were freed from side to the tubes or flasks by sterilized pipette or inoculation loop. e. The tube was kept in the refrigerator at 4 ºC - 8 ºC for overnight to separate the serum. f. Serum was carefully removed with the help of sterile syringe and needle and was centrifuged at 2000 rmp for 10 minutes for clarification. g. The serum was collected in sterilized vials. h. The serum was applied in water bath for 30 minutes at 50 ºC for destroying compliment. i. The prepared serum was stored at -20 ºC for further use.

Determination of Tri-iodothyronine (T_3), Thyroxine (T_4), Thyroid Stimulating Hormone (TSH) and Cortisol

T_3 is one of the two hormones secreted by the thyroid gland. The main part of the circulating T_3 is, however, produced by peripheral deiodination of thyroxine (T_4). T_4 is one of the two hormones secreted by the thyroid gland. T_3, T_4 and TSH were determined as per technique of IRMA Kit Protocol, described by Beijing North Institute of Biotechnology Co. Ltd. Cortisol is a steroid hormone, in the glucocorticoid class of hormones, and is produced in the adrenal cortex within the adrenal gland. It is released in response to stress and low blood glucose. Cortisol was determinedas per technique of ELISA Kit Protocol, described by Beijing North Institute of Biotechnology Co. Ltd which was performed by Bangladesh Nuclear Power Commission, Mymensingh.

Meat Quality Measurement

pH measurement

The pH of the meat samples was determined by adding 10 g sample with 50 ml distilled water and homogenizing it for 60 s in a homogenizer. The pH values were measured using a digital pH meter (Hanna, HI 9002, USA).

Drip loss

A sample weight of approximately 100 g were placed in the netting and then suspended in airtight plastic bag, ensuring that the sample does not make contact with the bag, or placed within the container on the supporting mesh and sealed. After a storage period (48 h) at 4 °C, the sample was immediately removed from the containers, gently dried on absorbent paper, and reweighed. Drip loss is expressed as a percentage of the initial weight. Percent drip loss was calculated using the formula

$$\text{Drip loss} = \frac{\text{initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Cooking loss

Cooking loss was determined by the weight difference in meat between before and after cooking relative to the weight of uncooked samples (Dominguez et al., 2015). After determination of thawing loss percentage, muscle was weighed (W_1) and transferred in water tight PVC-plastic bags before being boiled. Meanwhile, water was boiled to pre-set 71 °C using a water-bath (Personal-11 SM set, Taitec Corporation, Japan). The samples were then cooked to a core temperature of 71 °C for 1 h (AMSA, 2015). After cooking, meat samples were then removed from the water-bath, and cooled to room temperature (± 20 °C; measured using an analogue thermometer) under running water for 30 min. The meat was then taken from their bag, dried with paper towel and weighed (W_2).

$$\text{Cooking loss} = \frac{\text{initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Water holding capacity (WHC)

WHC was measured according to the methodology of Choi et al. (2018). Thawed samples (1 g each) were wrapped in absorbent cotton and placed in a 1.5 ml eppendorf tube. The tubes with samples were centrifuged in a centrifuge separator (H1650-W Tabletop high speed micro centrifuge) at 10,000 RPM for 10 min at 4º centigrade temperature, following which the samples were weighed. The WHC% of the sample is expressed as the ratio of the sample weight after centrifugation to the initial sample weight, using the following formula:

$$\text{Water holding capacity (\%)} = \frac{\text{(Weight of sample after centrifugation)}}{\text{(Weight of sample before centrifugation)}} \times 100$$

Color measurements

L*, a*, b* color coordinate (where L* measures relative lightness, a* relative redness and b* relative yellowness) values were measured on a freshly cut surface of the muscle (between 11th and 12th ribs) after a 45 min bloom time at room temperature (25 ± 3 °C) using Minolta CR300 Chroma meter (Minolta Co., Osaka, Japan) with illuminant D65 as light source. This instrument has a color-measuring area with a diameter of 1.1 cm and was calibrated using a Minolta calibration plate (L* = 97.59, a* = −5.00, b* = +6.76). Three measurements were taken, at different positions, across the surface of each raw meat sample and then mean values were used for statistical analysis.

Statistical Analysis

Statistical analysis system (SAS, 2015) was used. Data were first analyzed by a general linear model analysis of variance procedure and the means were compared using Duncan's least significance multiple-range test. All data are expressed in the form of mean ± standard error (SE, n=11). Differences were considered significant for values of P<0.05.

Results

Indigenous transport methods impact on hormonal responses

Figure 1 shows the results of tri-iodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH) and cortisol of lambs exposed to different indigenous transport methods. T_3 and T_4 values increased significantly and were different from the baseline values. TSH level was increased only by DT and had no change with the others. Cortisol was increased following DT and WT transport methods and returned to the baseline level at WTW_{HJ}.

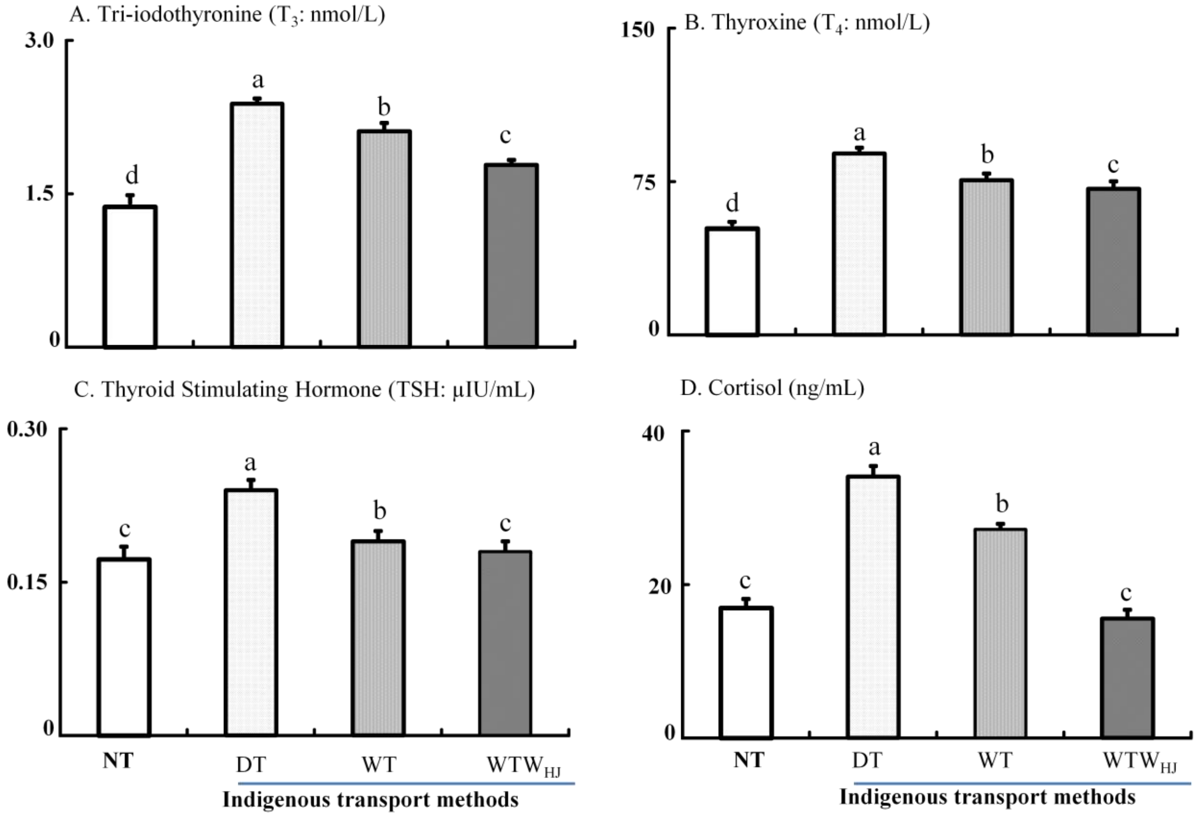

Fig. 1. Tri-iodothyronine (A), thyroxine (B), thyroid stimulating hormone (C), and cortisol (D) of lambs exposed to indigenous transport methods. Methods were NT (non-transport group), DT (3 h direct transport), WT (30 minutes walking before 3h direct transport), and WTW_{HJ} (30 minutes walking before 3 h direct transport afterwards again 30 minutes walking in human movement). Values represent the mean ± SE for 11 lambs in each treatment. $^{a\text{–}d}P<0.05$ compared among groups for different indigenous transport methods.

Indigenous transport methods impact on meat quality traits

Table 1 shows pH, drip loss, cooking loss, water-holding capacity, and CIE L*, a*, b* values in meat of slaughtered lambs of NT, DT, WT, and WTW_{HJ} groups. Lambs upon exposure to indigenous transport methods showed elevated ultimate pH values than non-transport lambs. Both drip and cooking loss was significantly lower in indigenous transport protocols as compared to the control. The opposite trend was observed in case of water-holding capacity parameter. CIE L*, b* and a* values were significantly altered by indigenous transport methods.

Table 1. Effect of indigenous transport methods on longissimus dorsi muscle quality characteristics of lamb

	Indigenous transport methods			
Trait	**NT**	**DT**	**WT**	**WTW_{HJ}**
Ultimate pH	5.62 ± 0.12[b]	5.95 ± 0.08[a]	6.10 ± 0.17[a]	6.32 ± 0.23[a]
DL (%)	5.7 ± 0.31[a]	5.21 ± 0.13[b]	4.88 ± 0.29[b]	4.65 ± 0.16[b]
CL (%)	36.25 ± 0.58[a]	25.76 ± 1.02[b]	23.11 ± 1.21[b]	19.53 ± 0.98[b]
WHC (%)	71.21 ± 1.21[b]	80.88 ± 0.85[a]	83.55 ± 1.36[a]	88.70 ± 0.22[a]
CIE L* (lightness)	38.03 ± 0.11[a]	29.62 ± 0.32[b]	30.21 ± 0.10[b]	28.75 ± 0.04[b]
CIE a* (redness)	17.9 ± 0.31[a]	14.30 ± 0.51[b]	13.63 ± 0.72[b]	11.82 ± 0.35[c]
CIE b* (yellowness)	7.7 ± 0.49[a]	7.4 ±0.03[a]	6.9 ± 0.93[b]	6.3 ± 0.62[b]

Drip loss (DL), cooking loss (CL), and water holding capacity (WHC) of lambs exposed to indigenous transport methods. Methods were NT (non-transport group), DT (3 h direct transport), WT (30 minutes walking before 3h direct transport), and WTWHJ (30 minutes walking before 3 h direct transport afterwards again 30 minutes walking in human movement). Values represent the mean ± SE for 11 lambs in each treatment. [a-c]$P<0.05$compared among groups for indigenous transport methods.

Discussion

The origin and quality of the produced meat is a rising concern in the global meat industry, especially for the third world countries. Moloney and McGee (2017) therefore suggested that farming should be focused with targeting the quantitative and qualitative traits of meat considering animal-influenced intrinsic factors, including gender, age, physiological status and non-animal-influenced extrinsic factors such as the environment and climate, nutrition, pre-slaughter management, and transport. Because the aforementioned traits determine the ultimate sensory features and consumer acceptance of the product (Skladanowska-Baryza et al., 2018; Hashem et al., 2013; Moniruzzaman et al., 2002a and 2002b; Sarkar et al., 2008). In addition, either short or prolonged period of stress before slaughter causes defects in meat like PSE (pale, soft and exudative) and DFD (dark, dry and firm), respectively (Greaser, 2001), and both of them are inducing significant losses to the meat industry. Pre-slaughter transportation acts as a short-term stress to the animals (Boukhris et al., 2017). As demand for meat products increases, transport of livestock for marketing are increasing from short-distance or long-distance sale yard, live animal market, abattoir, feedlot or pre-export assembly depot without considering welfare guidelines (Swanson and Morrow-Tesch, 2001). Such episode may incur physiological alternation as well as quality of the product. However, information concerning indigenous transport methods and their role of hormonal responses and meat quality of tender-age lambs in Bangladesh are limited. But this information is very much important for demonstrating animal’s response to stress (Azad et al., 2010; Rashid et al., 2013).

In this experiment, T_3 and T_4 values increased significantly and were different from the baseline values. TSH level was increased only by DT and had no change with the others. Cortisol was increased following DT and WT transport methods and returned to the baseline level at WTW_{HJ}.

The results of T_3 and T_4 hormones in the present experiment correspond with the statement reported by D’ Oliveira et al., 2014; Maheshwari et al., 2013; Mitchell et al.,1988; Fazio et al., 2015. Contrarily, Sejian et al. (2012) stated that Malpura ewes exposed to walking stress had a lower plasma T_3 and T_4 concentration. Salahuddin et al. (2019) found walking stress before and after transportation abated the stress to goats and could stabilize their physiological condition.The trend of elevated T_3 and T_4 in different indigenous transport results probably from a decreased peripheral deiodination (Kühn et al., 1987; Elnagar and Bech, 2000). The decreased plasma T_3 and T_4 level in control animals might be related to the effect of feed deprivation. It is well known that thyroid hormones play a crucial role in thermoregulation in animals, and plasma T_3 levels are positively correlated with heat production. Thyroid hormones accelerate the basal metabolic rate and oxidative metabolism by causing an increase in the mitochondria mass, mitochondria cytochrome content and respiratory rate. Circulating cortisol is the most predominant measure of stress experienced during transport. Plasma cortisol increases when lambs are exposed to stressful conditions due to short journeys (De la Fuente et al. 2010), long journeys (Tadich et al. 2009), and double transport on the same day (Miranda-de la Lama et al., 2010). In the current study, cortisol levels were higher in DT and WT lambs, suggesting that transport with higher vibrations and jolting can be an acute stress. Our results agree with Ruiz-de la Torre et al. (2001), who found higher levels of plasma cortisol in lambs transported on rough roads. Similarly, Hall et al. (1998) found higher cortisol levels on sheep transported on rough journeys (compared with smooth journeys). The relationship between cortisol and fatness is not a new finding. In humans, the increase in cortisol was observed to be caused by significant increase in fatness (Mormede et al., 2011). Cortisol regulates energy by selecting the right type and amount of substrate (carbohydrate, fat or protein) that is needed by the body to meet the physiological demands that is placed upon it. This is because, under stressful conditions, the upregulation of cortisol production favors protein hydrolysis for energy production through gluconeogenesis (Burdick et al., 2011). This implies that cortisol plays a role in the metabolic processes, carcass characteristics and meat quality.

Lactic acid and pH decline are interdependent during postmortem. Meaning that the higher of lactic acid accumulation, the lower of muscle pH. On the other hand, lactic acid production is dependent on glycogen reservoir of the muscle. If the animal has lower glycogen content in their muscle before slaughter, there will be lower lactic acid production, resulting higher muscle pH (Lawrie, 1998). Transportation stress reduced glycogen content in muscle of livestock and poultry (Boukhris et al., 2017; Toldra, 2018). Therefore, it is expecting that transported-lamb will exhibit higher muscle pH compared to non-transported group. Our findings correlate the above hypothesis. Because muscle pH did not decline as much as the NT lambs showed. Water is mainly fixed in protein in muscle. Postmortem metabolism can affect the functionality of meat proteins responsible for water-holding capacity (Swatland, 1993). Contractile proteins (myosin and actin) remain more active with high muscle pH. Under such state, there is a little chance to release water from the intercellular into the extracellular space and then onto the meat surface, resulting in higher water-holding capacity (WHC). Both high pH and WHC lead to lower cook losses and better protein functionality (Hedrick et al., 1989; Barbut, 1993; Lawrie, 1998). In the case of increased WHC, there have reduced amount of free water to release, results in lower drip loss. Lambs upon exposure to indigenous transport methods significantly decreased cooking loss compared to the non-transport lambs (Table 1). As decreased cooking loss is associated with higher water-holding capacity and high pH in meat (Bouton et al., 1971), the present study reflects the earlier statement (Table 1). The results of drip loss did not correspond with the result of Salahuddin et al. (2019). This might be due to age or species variation or intensity of the stress protocol.

Meat color is one of the most important criteria that consumers use to select meat (Warriss, 2000). According to Ngapo et al., (2004) consumers prefer normal-colored meat and mostly discriminate against meat that is either too pale or too dark.In the present study, indigenous transport methods showed significantly ($p < 0.05$) lower CIE L*, *a**, and *b** values in lambs. This result indicates that muscles from these groups were darker with less red and yellow than muscle from control lambs. Apple et al. (1995) showed that meat from stressed goat was darker than that from non-stressed goat.

Conclusion

In conclusion, the present study indicated that indigenous transport methods may able to disturb the hormonal functions and also to produce poor grade lamb.

Conflicts of Interest

The authors declare that there are no potential conflicts of interests.

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