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MUSCLE N^{τ}-METHYLHISTIDINE CONCENTRATIONS AND GLUCIDIC POTENTIAL IN TRANSPORTED CATTLE TREATED WITH ELECTROLYTES

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BACKGROUND

Transportation represents a significant stress for cattle due to a combination of several factors, such as fasting, mixing of unfamiliar animals, loading, length of transport and time in lairage. This stress due to transport can exert subsequent effects on the quantity as well as the quality of the meat produced. For example, Jones *et al.* (1992) showed that the weight loss observed in cattle as a result of transportation and lairage was comprised of tissue loss from the carcass as well as a reduction in the gastrointestinal contents. In addition, transport stress results in an increased incidence of dark-cutting meat (Tarrant, 1990).

These changes in carcass quality and yield reflect alterations taking place at the cellular level in the muscle. For example, an increased incidence of dark, firm and dry (DFD) meat is the result of stress-induced depletion of glycogen reserves (Tarrant, 1990). In the same way, weight loss from the carcass may be due to increased protein degradation as well as muscle tissue dehydration. Although the role of muscle protein degradation in the ageing of meat is widely known, few studies have examined factors which may alter protein degradation in the ante-mortem period, nor the subsequent effects of these alterations on meat yield and quality. However, we recently reported that levels of hormones and enzymes often associated with increased protein degradation were elevated following transport stress (Scott *et al.*, 1993).

If transport stress increased muscle protein degradation in addition to decreasing glycogen reserves, then a treatment able to counter these negative effects would be economically beneficial. Previous research from our laboratory has shown that electrolytes can be used to effectively counter transport stress. Schaefer *et al.* (1992) showed that providing an electrolyte drink to cattle in lairage could reverse some of the physiological effects of transportation stress and Jones *et al.* (1992) also showed that it was able to increase cold carcass weight.

OBJECTIVES

The overall goal of this study was to evaluate the effects of electrolyte treatment on muscle tissue metabolism in transported cattle. The first specific objective was to examine its effects on muscle glucidic potential, which is defined as the sum of glycogen, glucose, glucose-6-phosphate, and lactic acid (Charpentier, 1968). Although urinary excretion of N^{τ}-methylhistidine (N^{τ}-MH) can be used to estimate the muscle protein breakdown in cattle (Harris and Milne, 1981), we wanted to evaluate the possible effects of electrolyte treatment on the N^{τ}-MH content of muscle protein itself; this was the study's second specific objective.

METHODS

Twenty-eight bulls divided into 7 groups were used in the study. For each group, animals were initially loaded onto a trailer fitted with individual stalls with access to food and water. After 24 h in the trailer, cattle were unloaded and the food removed. Each bull received a drench of either water or a concentrated mixture of electrolytes (Nutricharge, Triple M Feeds, Red Deer, AB) before being loaded back onto the trailer and transported for 4 h. Following transport, they were held overnight in lairage in the trailer with access only to water. The next morning, cattle were slaughtered at the Lacombe Meat Research Centre abattoir.

At slaughter, two muscle cores were collected from the M. *Longissimus dorsi* (LD) of the carcass immediately post-exsanguination (time 0 post-mortem). After hide removal, approximately 15 min post-mortem, a muscle core was removed from the M. *Semimembranosus* (SM). After evisceration, approximately 30 min post-mortem, a muscle core was removed from the M. *Psoas major* (PM). In order to study the change in glucidic potential of the LD over time, core samples were also removed at 45 min, 3 h, and 24 h post-mortem. All muscle core samples were immediately frozen in liquid nitrogen and stored in whirl-packs at -35°C until analysed.

The muscle glucidic potential was determined using the LD core samples obtained at time 0 min, 45 min, 3h, and 24 h post-mortem. Frozen samples were pulverised in liquid nitrogen, followed by homogenisation of a 1g subsample in 5 ml cold 0.6 N $HCLO_4$. A 0.2 ml aliquot of the homogenate was analysed for glycogen as described by Dalrymple and Hamm (1973). The remaining homogenate was centrifuged for 20 min at 2000*g* at 2°C then neutralized as described by Dalrymple and Hamm (1973), except the centrifugation replaced the filtration step. The neutralized extracts were assayed for glucose as described by Dalrymple and Hamm (1973), for glucose-6-phosphate as described by Lang and Michal (1974), and for lactate as described by Gutmann and Wahlefeld (1974).

The N^{τ}-methylhistidine content of muscle protein (mmol g-1 protein) was determined using the LD, SM, and PM muscle core samples obtained from the carcass immediately post-mortem. Frozen muscle samples were pulverised in liquid nitrogen, then the ground muscle powder was subsampled for separate determinations of tissue protein content and of protein-bound N^{τ}-MH content . Tissue protein content was determined using the Hartree (1972) modification of the the Lowry method. Protein-bound N^{τ}-MH was analysed following precipitation of mixed muscle proteins with 10% $HCLO_4$, centrifugation for 10 min at 1700*g* and hydrolysation in 6.0 N HCl. N^{τ}-MH in the hydrolysate was analysed by HPLC using histidinol as an internal standard following derivatization with fluorescamine.

Data were subjected to an analysis of variance using a general linear model (SAS, 1989). For the analysis of the glucidic potential of the LD muscle, a split-plot model was used which included electrolyte treatment and breed as effects in the main plot, and sampling time in the subplot. For the analysis of the N^{τ}-methylhistidine content of muscle protein, a split-plot model was used which included electrolyte treatment and breed as effects in the main plot, and type of muscle in the subplot. For both analyses, treatment, breed, and the interaction between the two were tested against animal nested within treatment*breed as the error term.

RESULTS AND DISCUSSION

Concentrations of all the metabolites in the LD muscle involved in the determination of glucidic potential changed significantly with time post-mortem ($P<0.001$), but were not altered by electrolyte treatment prior to transport (Table 1), nor by any interactions between the main effects. Since the animals were in lairage overnight following transport, it is likely that any possible effects of the electrolyte treatment on glucidic potential were masked by the replenishment of glycogen stores in that intervening period. Perhaps the pre-transport electrolyte treatment would have more of an effect in animals slaughtered immediately after transport, as is the case in certain commercial abattoirs.

The mean N^{τ}-methylhistidine (N^{τ}-MH) content of muscle protein determined in the study (4.70 µmol N^{τ}-MH g^{-1} protein) is very close to the mean of 3.5 µmol N^{τ}-MH g^{-1} protein reported by Nishizawa *et al.* (1979) for Holstein steers. Few, if any, data have been published previously on the muscle protein N^{τ}-MH content in beef cattle. The N^{τ}-MH content of mixed muscle proteins was not affected by

electrolyte treatment, nor by the type of muscle studied (Table 2). These results provide valuable information about the validity of using urinary N^{τ}-MH excretion to estimate muscle protein degradation in transported and electrolyte-treated cattle. They verify that any alterations observed in N^{τ}-MH excretion will be due to changes in its release as a result of protein degradation, rather than to variations in its initial concentration in muscle protein.

CONCLUSIONS

The glucidic potential of LD muscle was unchanged by pre-transport electrolyte treatment, although the pre-slaughter time in lairage may have allowed a replenishment of glycogen stores. N^{τ}-Methylhistidine content of mixed muscle protein in the LD, PM, and SM muscles was also unchanged by pre-transport electrolyte treatment. This implies that it will be valid to use the values determined in the present study to evaluate the effects of electrolytes on muscle protein degradation in cattle, as estimated from urinary N^{τ}-MH excretion.

LITERATURE CITED

CHARPENTIER, J. 1968. Breakdown of glycogen post-mortem in the longissimus dorsi muscle of pigs. Ann. Zootech. 17:429-443.

DALRYMPLE, R.H., AND HAMM, R. 1973. A method for the extraction of glycogen and metabolites from a single muscle sample. J. Fd. Technol. 8: 439-444.

GUTMANN, I., AND WAHLEFELD, A.W. 1974. L-(+)-lactate: Determination with lactate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis*, 2nd ed., vol. 3, H.U. Bergmeyer, ed. Academic Press, New York, N.Y.

HARRIS, C.I., AND MILNE, G. 1981 The urinary excretion of N^{τ}-methylhistidine by cattle: validation as an index of muscle protein breakdown. Br. J. Nutr. 45: 411-422.

HARTREE, E.F. 1972. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48: 422-427.

JONES, S.D.M., SCHAEFER, A.L., AND TONG, A.K.W. 1992. The effects of fasting, electrolyte supplementation, and electrical stimulation on carcass yield and meat quality in bulls. Can. J. Anim. Sci. 72: 791-798.

LANG, G., AND MICHAL, G. 1974. D-glucose-6-phosphate and D-fructose-6-phosphate. In: *Methods of Enzymatic Analysis*, 2nd ed., vol. 3, H.U. Bergmeyer, ed. Academic Press, New York, N.Y.

NISHIZAWA, N., TOYODA, Y., NOGUCHI, T., HAREYAMA, S., ITABASHI, H., AND FUNABIKI, R. 1979. N^{τ}-Methylhistidine content of organs and tissues of cattle and an attempt to estimate fractional catabolic and synthetic rates of myofibrillar proteins of skeletal muscle during growth by measuring urinary output of N^{τ}-methylhistidine. Br. J. Nutr. 42: 247-252.

SAS INSTITUTE, INC. 1989. *SAS/STAT User's Guide*. Version 6, 4th edition. SAS Institute, Cary, NC.

SCHAEFER, A.L., JONES, S.D.M., TONG, A.K.W., YOUNG, B.A., MURRAY, N.L., AND LEPAGE, P. 1992. Effects of post-transport electrolyte supplementation on tissue electrolytes, hematology, urine osmolality and weight loss in beef bulls. Livest. Prod. Sci. 30: 333-346.

SCOTT, S.L., SCHAEFER, A.L., JONES, S.D.M., MEARS, G.J., AND STANLEY, R.W. 1993. Stress indicators and lean tissue yield in transported cattle treated with electrolytes. Proc. 39th ICMST, Calgary, AB, Canada, August 1-6.

TARRANT, P.V. 1990. Transportation of cattle by road. Appl. Anim. Behav. Sci. 28: 153-170.

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DATA

Table 1: Effects of electrolyte treatment on glycogen, glucose, glucose-6-phosphate and lactate concentrations ($\mu mol\ g^{-1}$) and on glucidic potential of M. *Longissimus dorsi* from transported cattle.

Treatment	Sham Drench				Electrolyte Drench				Probability	
Time	0 min	45 min	3h	24 h	0 min	45 min	3h	24 h	Treatment	Time
Glycogen	50.9 ±2.4	50.4 ±2.4	46.0 ±2.4	8.48 ±2.4	58.6 ±2.2	54.0 ±2.2	45.0 ±2.2	12.9 ±2.2	0.4223	0.0001
Glucose	0.69 ±0.2	0.38 ±0.2	0.52±0.2	5.11±0.2	0.78 ±0.2	0.63 ±0.2	0.60 ±0.2	4.45 ±0.2	0.7532	0.0001
Glucose-6-P	0.08 ±0.1	0.05 ±0.1	0.05 ±0.1	2.15 ±0.1	0.10 ±0.1	0.06 ±0.1	0.14 ±0.1	1.96 ±0.1	0.8108	0.0001
Lactate	17.1 ±2.7	18.1 ±2.7	35.9 ±2.7	79.4 ±2.7	14.8 ±2.5	17.8 ±2.5	33.6 ±2.5	72.8 ±2.5	0.5502	0.0001
Glucidic Potential	60.2 ±2.4	59.9 ±2.4	64.6 ±2.4	55.4 ±2.4	66.9 ±2.3	63.6 ±2.3	62.6 ±2.3	55.7 ±2.3	0.6185	0.0031

Table 2: Effects of electrolyte treatment on mixed muscle protein N^{τ}-methylhistidine (N^{τ}-MH) content ($\mu mol\ g^{-1}$ protein) in three muscles from transported cattle.

Treatment	Sham Drench			Electrolyte Drench			Probability	
Mucle	LD	PM	SM	LD	PM	SM	Treatment	Muscle
N^{τ}-MH content	5.12 ± 0.42	5.07 ± 0.35	4.83 ± 0.29	4.76 ± 0.31	4.96 ± 0.27	4.47 ± 0.27	0.3892	0.4383