BOVINE MUSCLE GLYCOGEN CONCENTRATION IN RELATION TO DIET, SLAUGHTER AND ULTIMATE BEEF QUALITY

Kaisa Immonen

ACADEMIC DISSERTATION

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Supervisor and *Custos*: Prof. Eero Puolanne
Department of Food Technology
Meat Technology
University of Helsinki
Helsinki, Finland

Reviewers: Prof. Kerstin Lundström
Department of Food Science
Swedish University of Agricultural Sciences
Uppsala, Sweden

Prof. Reeta Pösö
Department of Basic Veterinary Sciences
Veterinary Biochemistry
University of Helsinki
Helsinki, Finland

Opponent: Dr. P. Vivion Tarrant
The National Food Centre
Dublin, Ireland

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To the true motivators of my life:

My mother Pirkko and my father Pauli,

My beloved husband Antti,

And my dear sons Erkko and Asse.

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TIIVISTELMÄ, FINNISH SUMMARY

Tämän työn tarkoituksena oli tutkia: 1) naudan lihasten glykogeenikonsentraatiota kasvatustilalla, teuraskuljetuksen jälkeen sekä *rigor mortis*'n muodostumisen jälkeen suhteessa eri tyyppisiin ruokintoihin, 2) naudanlihan loppu-pH:n suhdetta jäännösglykogeenikonsentraatioon, sekä 3) jäännösglykogeenipitoisuuden vaikutuksia naudanlihan fysikaaliseen ja aistinvaraiseen laatuun. Työn tavoitteena oli tuottaa uutta tietoa integroitua ja kokonaisvaltaista naudanlihan laadunhallintaa varten. Tähän työhön liittyviä tutkimuksia tehtiin kahdessa maassa: USA:ssa, Wisconsinin yliopistossa, Madisonissa, sekä Suomessa, Helsingin yliopistossa.

Näytteenottokohta vaikutti naudan *longissimus thoracis et lumborum* (LTL) lihaksesta mitattavaan glykogeenikonsentraatioon. Läheltä 10. kylkiluuta otetuissa näytteissä oli vähemmän glykogeenia ($p \leq 0.05$) kuin näytteissä, jotka oli otettu 11. kylkiluun kaudaaliselta puolelta. Naudan lihaksen toistuvaa näytteenottoa voidaan varovasti pitää oikeutettuna sillä perusteella, että lihaksensisäinen hajonta oli 11.6% ja glykolyyttisen potentiaalin laskeminen poisti sijaintien välisten erojen merkitsevyyden. Saadut tulokset korostavat kuitenkin ko. lihaksen metabolisesti heterogeenistä luonnetta ja osoittavat, että LTL lihaksen toistuvassa näytteenotossa täytyy harjoittaa erityistä varovaisuutta ja harkintaa, etenkin jos biokemiallisia määrityksiä tehdään tuoreena pakastetuista lihasnäytteistä.

Naudan lihaksen glykogeenikonsentraatio ei ollut ruokinnalla helposti manipuloitavissa. Etenkään lihakset, joiden glykogeenipitoisuus oli jo alussa suuri, eivät reagoineet ruokinnan energiapitoisuuden muuttumiseen. Mitä enemmän glykogeenia oli lähtötilanteessa, sitä vähemmän glykogeenia syntetisoitui lisää ruokintojen vaikutuksesta ($r = -0.678$ (FIN), $r = -0.527$ (USA), $p<0.0001$). Toisaalta, naudat kykenivät lisäämään ja ylläpitämään lihasten glykogeenipitoisuutta jopa energiapitoisuudeltaan matalalla täyssäilörehuruokinnalla. Lisäksi lihasten glykogeenipitoisuus laski hyvin vähän, kun kuukauden energiapitoisuudeltaan korkealla rehulla ruokitut naudat vaihdettiin matalaenergiaruokinnalle. Luurankolihasten glykogeenipitoisuudelle näyttää olevan luonteenomaista se, että levossa ja stressittömissä olosuhteissa se pyrkii pysymään vakiona myös suhteellisen vaatimattomalla ruokinnalla.

Maissi lisäsi härkien ja sonnien lihasten glykogeenipitoisuutta merkitsevästi paremmin kuin alfa-alfa säilörehu, kun lihasten glykogeenipitoisuus oli ensin laskettu adrenaliinilla keskimäärin tasolle 50 mmol/kg. Urosstatuksella, sonni versus härkä, ei ollut johdonmukaista vaikutusta glykogeenipitoisuuteen eikä ruokinnan vaikutukseen glykogeenipitoisuuteen. Korkeaenergiaruokinta näytti kuitenkin suojaavan nautoja potentiaalisilta glykogeenia kuluttavilta stressitekijöiltä, kuten kylmiltä ja kuumilta säiltä, sekä toisaalta teuraskuljetukselta. Suomessa korkeanergiaruokitut sonnit menettivät glykogeenia teuraskuljetuksen aikana 7 ± 4.0 (se) mmol/kg keväällä (viileä ilma) ja 23 ± 3.9 mmol/kg kesällä (helle), kun taas mataenergiaruokittujen sonnien glykogeenipitoisuus laski teuraskuljetuksessa 16 ± 3.8 mmol/kg keväällä ja 33 ± 4.1 mmol/kg kesällä. Nämä vaikutukset heijastuivat aina lopullisiin pH-arvoihin (5.69 ± 0.03 (korkeaenergia); 5.93 ± 0.03 (matalaenergia)) ja jäännösglykogeenipitoisuuksiin saakka. Ainakin Suomen olosuhteita ajatellen voidaan tehdä se johtopäätös, että lyhytkin (2 vk) teuraskuljetukseen asti kestänyt loppukunnostus paljon energiaa sisältävällä väkirehulla on toteuttamisen arvoinen toimenpide, sillä sen suojaavat vaikutukset suuntautuvat selvästi stressitilanteisiin liittyvän glykogeenikatabolian ja vaarana olevan pH:n nousun hillitsemiseen sekä näin ollen tervalihaisuuden ehkäisemiseen.

Naudanlihan loppu-pH:n ja jäännösglykogeenikonsentraation riippuvuus oli epälineaarinen. Jäännösglykogeenipitoisuuden vaihtelu oli valtavan suuri, 10–83 mmol/kg, ja loppu-pH-arvosta riippumaton normaalin pH:n alueella ($pH \leq 5.75$). Matalien jäännösglykogeenipitoisuuksien voidaan katsoa toimivan indikaattoreina teurastusta edeltäneestä glykogeenikataboliasta.

Jäännösglykogeenilla oli monia itsenäisiä, joskin merkitykseltään vaatimattomia vaikutuksia pH-arvoltaan normaalin naudanlihan fysikaaliseen ja aistinvaraiseen laatuun. Jäännösglykogeenipitoisuus vaikutti lihan vedenpidätyskykyyn, mm. valuman ja sulatushävikin muodossa, kuten myös tuoreen lihan punaisuuteen, leikkausvoimaan, sekä pihvin värin b*-arvoon. Ko. vaikutuksista valumaan, leikkausvoimaan ja pihvin b*-arvoon kohdistuneet olivat luonteeltaan positiivisia. Kaiken kaikkiaan jäännösglykogeenin laatuvaikutuksista voidaan todeta, että erittäin paljon tai vain vähän glykogeenia sisältäneet näytteet poikkesivat laadultaan hieman toisistaan. Sitä vastoin keskimääräisen runsaasti glykogeenia sisältäneiden näytteiden laatukäyttäytyminen oli jommankumman edellisen kaltainen. Jäännösglykogeenin negatiiviset vaikutukset laatuun, kuten sulatus- ja paistohävikkiin, pihvin mehukkuuteen sekä tuoreen lihan punaisuuteen, eivät olleet sitä suuruusluokkaa, että ne aiheuttaisivat mitään toimintatarpeita.

Immonen, K. 2000. Bovine muscle glycogen concentration in relation to diet, slaughter and ultimate beef quality. Dissertation. EKT series 1203. University of Helsinki. Department of Food Technology. 61 + 36 pp.

ABSTRACT

The aims of this study were to investigate: 1) bovine muscle glycogen concentration on-farm, after transportation to slaughter and after *rigor mortis* development in relation to various types of diets, 2) the relationship between the ultimate pH and residual glycogen concentration, and 3) the effects of residual glycogen on the physical and sensory quality of beef. The ultimate goal of this work was to gain additional knowledge and tools for integrated beef quality control. The experiments of this study were conducted in two countries: USA, at the University of Wisconsin-Madison, and Finland, at the University of Helsinki.

Glycogen concentration in bovine *longissimus thoracis et lumborum* (LTL) muscle was affected by *in vivo* sampling location. Cranial locations, which were close to the 10th rib, had less ($p \leq 0.05$) glycogen than locations in the mid-section and caudal to the 11th rib. Present results substantiate the metabolic heterogeneity of the LTL muscle, and indicate that special care has to be taken in exercising repeated sampling, particularly, if biochemical analyses are to be performed on fresh-frozen muscle specimen. However, because the overall variation within the LTL muscle was not more than 11.6% and also because the calculation of glycolytic potential (GP) rendered locational concentration differences insignificant, the use of LTL in experiments requiring repeated sampling can be cautiously encouraged.

Bovine LTL and *gluteus medius* (GM) muscle glycogen could not be easily manipulated with diets. Initially high muscle glycogen concentrations were not readily affected by the change in the energy density/energy content of the feed. The more there was glycogen initially the less glycogen increased ($r = -0.678$ (FIN), $r = -0.527$ (USA), $p<0.0001$). Persistence seems to be a characteristic of skeletal muscle glycogen even when a relatively low energy diet is consumed, since cattle were able to gain and maintain high glycogen concentrations with a low energy diet of 100% silage. Furthermore, muscle glycogen decreased minimally when low energy diet was provided following a month on high-energy diet. Corn was superior to alfalfa haylage in increasing glycogen content in the muscles of steers and bulls, when the initial glycogen content was depleted to 50 mmol/kg with one subcutaneous injection of adrenaline. Male status, i.e., bull versus steer, did not consistently affect glycogen concentration nor responsiveness to dietary treatment. However, high-energy diet seemed to protect cattle from potentially glycogen-depleting stressors such as very low and high temperatures as well as transportation to slaughter. In Finland, the cattle on high energy lost 7 ± 4.0 (se) mmol/kg and 23 ± 3.9 mmol/kg during transportation in spring (cool weather) and in summer (hot weather), respectively, whereas the cattle on low energy diet lost 16 ± 3.8 mmol/kg in spring and 33 ± 4.1 mmol/kg in summer. These effects of diet were reflected all the way to ultimate pH values (5.69 ± 0.03 (high energy); 5.93 ± 0.03 (low energy)) and residual glycogen concentrations. As far as the beef production in Finland is concerned, it can be concluded that even a short finishing of two weeks with a concentrate-based high-energy diet is well worth applying, because its clearly protective effects were directed at glycogen depletion and elevation of pH. Thus, it is one of the many appropriate measures to take in the fight against bovine dark-cutting, which is a well-known quality problem causing reduced shelf life and unattractively dark color.

The relationship between pH and residual glycogen concentration was curvelinear. At normal pH values ($pH \leq 5.75$) the variation in residual glycogen concentration was enormous, from 10 to 83 mmol/kg, and independent of ultimate pH. Low residual carbohydrate serves as an indicator of diminished glycogen content at the time of slaughter.

The independent effects of residual glycogen concentration on the physical and sensory quality of normal-pH-beef were somewhat numerous but quite modest. The water holding capacity, characterized e.g., with drip loss, and weight loss in thawing, was affected as were fresh meat redness (Hunter's a* value), shear force, and Hunter's b-value of the steak, of which the effects on drip loss, shear force, and b*-value of the steak color can be considered positive. Of all the quality effects of residual glycogen concentration, it can be concluded that it is mainly the beef having either low or very high glycogen concentration that behave slightly differently from each other, the beef having intermediate (somewhat high) residual glycogen concentration mostly follows the pattern of one or the other. The negative effects of high residual glycogen on beef quality ie., on weight loss in thawing and frying, sensory juiciness, and fresh meat redness, are not of the magnitude to call for action.

Key words: bovine, diet, *gluteus medius*, glycogen, *longissimus thoracis et lumborum*, meat quality, pH_u, residual glycogen

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Helsinki, September 2000

Kaisa Immonen

ABBREVIATIONS

adr	adrenalin
AH	alfalfa haylage
AMP	adenosine monophosphate
B1-B6	biopsies obtained at different stages of the experiment
BC	buffering capacity
C	corn
CV	coefficient of variation
dGly-diet	change in glycogen concentration while on diet (paper IV)
dGly-pm	loss of glycogen after slaughter (paper IV)
dGly-trnsp	loss of glycogen concentration between farm and slaughter (paper IV)
DC	dark-cutting
DM	dry matter
E	energy (as in High E)
EUROP	European Community scale for the classification of carcasses of adult bovine animals
EXP	experiment
Ext	extensive housing
FG	fast-twitch, glycolytic muscle fibre
FOG	fast-twitch, oxidative, glycolytic muscle fibre
Gly-diet	glycogen concentration after 14 days on diet (paper IV)
Gly-kill	calculated glycogen concentration at the time of slaughter (paper IV)
Gly-resid	residual glycogen concentration (paper IV)
Gly-rest	resting muscle glycogen concentration (paper IV)
GM	gluteus medius muscle
GP	glycolytic potential
HK	*hexokinase*
In-pen	animal´s rank order in sampling within its penmates
Int	intensive housing
kgF	force in kilograms needed to shear meat with Instron
Kill-gly	calculated glycogen concentration at the time of slaughter (II) = Gly-kill (IV)
LA	lactic acid
LL	*longissimus lumborum* muscle
LTL	*longissimus thoracis et lumborum* muscle (also referred to as *longissimus dorsi)*
ME	metabolizable energy
MG	macroglycogen ($M=10^6 - 10^7$)
NAD^+	nicotinic adenine dinucleotide
$NADP^+$	nicotinic adenine dinucleotide phosphate
N/A	not applicaple
PCA	perchloric acid
PG	proglycogen (M=400000)
pH_u	ultimate pH
PM	*psoas major* muscle
p.m.	*postmortem* ie. after death
QC	quality control
r_{min}	smallest coefficient of correlation
Res-gly	residual glycogen concentration (paper II) = Gly-resid (IV)
SD	standard deviation (sd)
SE	standard error (se)
SM	*semimembranosus* muscle
SO	slow-twitch, oxidative muscle fibre
SS	*supraspinatus* muscle
ST	*semitendinosus* muscle
Temp-1	first temperament recording at sampling
VFA	volatile fatty acid
WHC	water holding capacity
X	mean

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original articles, which are referred to by their Roman numerals I - V.

I Immonen, K., Kauffman, R. G., Schaefer, D. M. and Puolanne, E. 2000. Glycogen concentration in bovine longissimus muscle. Meat Science 54, 163-167.

II Immonen, K., Schaefer, D. M., Puolanne, E., Kauffman, R. G. and Nordheim, E. V. 2000. The relative effect of dietary energy density on repleted and resting muscle glycogen concentrations. Meat Science 54, 155-162.

III Immonen, K. and Puolanne, E. 2000. Variation of residual glycogen-glucose concentration at ultimate pH values below 5.75. Meat Science 55, 279-283.

IV Immonen, K., Ruusunen, M., Hissa, K. and Puolanne, E. 2000. Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. Meat Science 55, 25-31.

V Immonen, K., Ruusunen, M. and Puolanne, E. 2000. Some effects of residual glycogen concentration on the physical and sensory quality of normal pH beef. Meat Science 55, 33-38.

CONTENTS

INTRODUCTION

Glycogen has a crucial role as a substrate for energy metabolism of living muscle as well as the muscle turning into meat after the animal has been slaughtered. Muscle glycogen concentration at the time of slaughter is one of the most important factors affecting ultimate meat quality.

Glycogen is a branched polysaccharide of α-D-glucose units. Most glucose residues are linked together by α-1,4-glycosidic bonds. Branches are generated by α-1,6-glycosidic bonds (Beitz, 1993). The bush-like glycogen molecules have molecular weights in the millions. In skeletal muscle, glycogen forms subcellular particles designated as "glycogen particles". These small particles contain glycogen and high concentrations of the major enzymes that synthesize and break down glycogen. Glycogen can be viewed as stored glucose that is rapidly mobilized and used to produce energy (Bechtel and Best, 1985). The enzymes involved in the hydrolysis of glycogen are *phosphorylase*, mobilizing the long straight chains as glucose-1-phosphate; *transferase*, transferring a chain of three glucose residues at the end of another chain; and *amylo-1,6-glucosidase*, cleaving the last remaining glucose residue from the 1,6-linkage as free glucose (Lehninger, 1993).

The glycogen content in skeletal muscle is approximately 1.5-1.8 % (Monin, 1981; McVeigh and Tarrant, 1982) but will vary with exercise and nutritional status (Bechtel and Best, 1985). The breakdown of glycogen can be triggered by increased circulating adrenaline or by strenuous muscular activity. In addition, glycogen is slowly depleted during starvation. After the death of the animal when oxygen is no longer available, the *postmortem* breakdown of muscle glycogen yields lactic acid, the accumulation of which is responsible for the normal low pH of meat (5.5), the essential prerequisite for good meat quality.

A low at-death glycogen content results in a raised ultimate pH that, in extreme cases, can be near 7 (Howard and Lawrie, 1956). Meat is referred to as having a quality problem called dark-cutting if the ultimate pH is 6.0 or above. Any behavior and environmental circumstances that trigger one or more of the glycogen breakdown mechanisms in a living animal will cause dark-cutting if the stress is allowed to persist for sufficient time (Tarrant, 1989a).

The incidence of dark-cutting beef varies widely. In the case of young dairy bulls in Finland, it used to be a serious problem (> 25% dark-cutters), until single pens were implanted to most slaughter houses in Finland during the 1980's lowering the incidence to 1-5% (Puolanne and Aalto, 1981). The incidence of dark-cutting has decreased, but the proportionate amount of elevated pH values, ie., 5.7–5.9, has remained rather high (Honkavaara, 2000, personal communication).

The properties of meat that are of most interest to the consumer, e.g., tenderness, juiciness and flavor, are strongly affected by perimortal treatments: the physical conditions that are imposed on the animal in the last few days of life and on the carcass in the first few hours post-mortem (Marsh, 1993). Domestic animals often experience physiological insults when they are transported and handled. Transport and handling stress reduces carcass yield, degrades well-being and meat quality (Lahucky *et al.*, 1999). Indeed, in beef, it is stress rather than under-nutrition that lowers the glycogen content and consequently elevates ultimate pH; it is thus the prevention of stress (particularly in the few days preceding slaughter and during transportation to slaughter) that must be the goal if dark-cutting is to be eliminated. It is obvious, though, that animals vary widely in their individual abilities to resist stress. (Marsh, 1993).

Stress may be climatic, such as intensive cold or heat; nutritional, due to feed deprivation; social, resulting from a low rank in the pecking order or internal, due to some physiological disorders, pathogens or toxins (Hafez, 1968). From this point of view, the “stressed” animal is considered abnormal and the condition regarded as undesirable (Stott, 1981).

Even though great attempt has been made to minimize animal stress during transportation to slaughter, farmers themselves have little direct control over the stressors that are presented to the animals after they leave the farm. However, they could implement on-farm practices aimed at minimizing glycogen depletion, or alternatively at providing a ‘glycogen buffer’ prior to trucking (Lambert *et al.*, 1998).

An attempt to create the glycogen buffer could be made by modifying the pre-slaughter feeding practices to supply appropriate gluconeogenic precursors, especially propionic acid, (Lister, 1989) to maximize the availability of blood glucose for cattle. What complicates this issue, is that there may be substantially less glucose available for ruminants than for non-ruminants on a high-carbohydrate or any diet (Lindsay, 1981).

Muscle glycogen concentration as well as residual glycogen concentration of ruminant muscles, as well as the ultimate pH of meat have, however, been successfully manipulated with dietary energy modifications (Pethick *et al.*, 1994; Pethick and Rowe, 1996). Therefore, it seems clear that this research area is yet far from completely understood as far as the metabolic events and their control mechanisms associated with the ruminant muscle glycogen, as well as its effects on ultimate meat quality are concerned.

REVIEW OF THE LITERATURE

Characteristics of bovine carbohydrate metabolism

Ruminants, so named because they ruminate (chew the cud), have a stomach that consists of a nonsecretory forestomach and a secretory stomach compartment (the abomasum). The forestomach consists of three compartments (the rumen, the reticulum, and the omasum) and serves as fermentation vat for the microbiological fermentation of the ingesta, mainly by hydrolysis and anaerobic oxidation. The fermentation end products (mainly volatile fatty acids (VFA)) that the ruminant absorbs and uses as its prime metabolic substrates are quite different from the end products of carbohydrate digestion (mainly glucose) in nonruminants. The abomasum, like the stomach of nonruminant animals, is largely concerned with the hydrolysis of protein by pepsin in an acid medium. The forestomach system allows the ruminant the use of diets that may be too fibrous for nonruminant animal, confers the ability to break down cellulose and allowing cellulose, itself the most abundant carbohydrate form present in the plant, to become a major nutrient, as well as allows the synthesis of high-biological-value microbial protein from low-biological-value plant protein, from dietary nonprotein nitrogen, and from recycled nitrogenous metabolic end products (e.g., urea). The fermentative end products of all carbohydrates are mainly acetic, propionic, and butyric acids (Leek, 1993).

Absorption of monosaccharides resulting from digestion of dietary carbohydrates constitutes a major, though variable, source of blood glucose in nonruminants. Although only small amounts of glucose are absorbed in most dietary situations in ruminants, glucose availability to ruminant tissues as measured by isotope dilution was shown to be substantial, indicating that gluconeogenesis is a major metabolic activity in both fed and fasted states (Annison and Bryden, 1999). Blood glucose level is thus maintained with endogenous synthesis of glucose in the liver from glucogenic compounds, such as glycogen, glucogenic amino acids, and glycerol, and, additionally, propionate in ruminants and pre-ruminants, such as the horse. Not surprisingly, the glucose production is linearly related to the availability of its precursors in plasma (Lindsay, 1978). The role of glucose in ruminant metabolism was recognized by D. B. Lindsay who concluded that glucose requirements in ruminants are similar in magnitude to those of non-ruminants (Lindsay, 1959). In ruminant animals, gluconeogenesis is of continual importance (Lindsay, 1978) because most dietary carbohydrates are fermented primarily to VFAs in the alimentary tract, and the propionate and the lesser amounts of valerate contribute to about half of the glucose synthesis in the liver (Bergman, 1973; Lindsay, 1978). Glucose availability for ruminants has thought to be limited because their blood glucose does not increase significantly after a meal (Lindsay, 1978). Furthermore, the mean blood glucose concentration of cows and mature sheep is only 2.2–4.4 mmol/l compared to pigs (4.4–6.7 mmol/l), horses (3.3–6.1 mmol/l) or dogs (3.9–6.7 mmol/l) (Swenson, 1993). However, Ballard *et al.* (1969) concluded that the rate of glucose production was approximately proportional to a power of ($W^{0.8}$) of body-weight and that ruminants and non-ruminants showed little difference in this respect.

On certain diets, particularly those containing ground corn or sorghum, appreciable amount of starch escape rumen fermentation so that up to 15–20% may be digested in the small intestine (Lindsay, 1981). In this case glucose is absorbed as such and contributes directly to the level of glucose in blood. For the diets, which contain ground corn, the glucose requirement could possibly be met by that absorbed from small intestine. Where the glucose supply is in any way precarious it would seem that the inclusion of ground corn in the diet might constitute a useful precaution (Lewis and Hill, 1983). However, even on diets containing high amounts of

concentrate, the absorption of glucose from the gut accounts for less than one-third of the whole-body glucose turnover (Bergman, 1973; van der Walt *et al.*, 1983).

In many species, glucose is recognized as the major source of glycerol used in triacylglycerol synthesis, and glucose oxidation provides much of the energy required for fatty acid synthesis (Vernon, 1981). In cattle fed grain, there is little uptake of glucose in the adipose tissue (Reynolds *et al.*, 1988), suggesting that under normal feeding conditions, the contribution of glucose to fatty acid synthesis is low. The short-chain volatile fatty acids probably represent two-thirds of the energy supply of the ruminant and are utilized in many ways that spare or form glucose. Acetic and butyric acids, on the other hand, do not give rise to blood glucose (Lewis and Hill, 1983).

It may be relevant that insulin secretion in ruminants is stimulated not only by glucose but also by several volatile fatty acids (propionate, butyrate, isovalerate and valerate) (Horino *et al.*, 1968; Brockman, 1982). This insulin-stimulating effect of propionate seems odd, though, since insulin is an effective inhibitor of hepatic gluconeogenesis (Lehninger, 1993), by which propionate is converted to glucose. In fact, Pösö and Hyyppä (1999) showed that propionic acid given to horses by naso-gastric tube post-exercise prevented any rise in insulin concentration. It may also be relevant, that the molar ratio of insulin:glucagon in ruminants is approximately 2, whereas for monogastric animals consuming high carbohydrate diets this value is nearer 100 (Lister, 1989). Glucagon stimulates the glycogenolysis and more importantly, the gluconeogenesis in the liver (Lehninger, 1993), and thus, increases the hepatic output of glucose, which is of much greater importance to ruminants than to monogastrics in the homeostasis of blood glucose.

Other dietary constituents also contribute carbon for the ruminal VFA synthesis. For example, when cellulose rather than starch is the major dietary carbohydrate for cattle, acetate is the major VFA produced. Increasing the proportion of starch will increase ruminal production of propionate and valerate and decrease production of acetate and butyrate (Beitz, 1993). Rumen fermentation may be shifted towards more propionic acid when diets with a high concentration of degradable starch are used (Fiems *et al.*, 1999). Clearly the proportions of different VFAs will vary with the quantity and quality of food consumed and the physiological status of the animal (Annison and Bryden, 1999).

Acetate is a major source of energy. It has been demonstrated to have relatively high contribution to total oxidative metabolism in sheep (Annison and Lindsay, 1961). Acetate is rapidly metabolized by the body (Brockman, 1993), and its uptake by muscles is increased by insulin (Knowles *et al.*, 1974). About 26% of respiratory CO_2 of sheep is derived from acetate (Pethick *et al.*, 1981).

It is well recognized, however, that the carbohydrate economy of the ruminant is vulnerable and changes in an animal's diet or demand for carbohydrate may alter carbohydrate balance in a devastating fashion (Lister, 1989). Acute and chronic acidosis, conditions that follow ingestion of excessive amounts of readily fermented carbohydrate, are prominent production problems for ruminants fed diets rich in concentrate (Owens *et al.*, 1998). Rapidly degradable carbohydrate may provoke acidosis (Fiems *et al.*, 1993).

In the study of Fiems *et* al. (1999) dietary starch characteristics, ie., ingested levels, and degradability, did not modify blood concentrations of lactate and glucose, but the urea concentration was higher for the cattle consuming high amounts of starch with the highest

degradability. Preference for the offered silage was different between groups. Animals consuming high amount of starch with low degradability preferred to eat the concentrate before the silage, whereas the silage was mostly eaten before the concentrate by animals consuming high amount of starch of high degradability. It was concluded that diets having large amounts of highly digestible fiber or rapidly degradable starch, may impair intake, growth rate and some meat quality parameters, when both feeds are fed separately (Fiems *et al.*, 1999).

Glycogen in bovine muscle

Concentration and metabolism at resting state

Although muscle in general can utilize both carbohydrates and lipids, most of its energy at rest is derived from the oxidation of circulating free fatty acids (Lindsay, 1981) and no net glycogen consumption can be measured (Tarrant, 1989a). As metabolic demand increases, carbohydrates are more readily utilized (glycogen breakdown and glucose transport). Ruminant muscles differ from those of monogastrics to some extent, since the main metabolic substrate provided by ruminants' digestive processes is acetate, which is taken up from the blood by muscles as well as adipose tissue and oxidized for energy as such (Vernon and Peaker, 1983), and may account for 35-40% of oxygen consumption (Lindsay, 1981). In man, acetate is likely to be significant as a substrate only following alcohol ingestion (Lindsay, 1981). Skeletal muscles are also capable of deriving considerable amounts of their chemical energy needs from ketone bodies, and thus, are less dependent upon blood glucose than e.g., the brain (Beitz, 1993). This is especially relevant in ruminants for which keto acids are constantly available through conversion of butyric acid in the rumen epithelium, whereas in monogastrics keto acids are produced and ketosis of varying intensity induced by starvation, high-fat or low-carbohydrate diets, impaired liver function, anesthesia, and endocrine disorders, such as diabetes mellitus (Bergman, 1993). Ruminant muscles get nearly half of their energy from ketones and acetate (Lindsay, 1981).

In resting cattle, muscle glycogen is restored primarily by synthesis from blood glucose and used stingily in metabolic processes. The average concentration glycogen is 80-100 mmol/kg (Table 1). Not all muscle glycogen is synthesized from glucose originating directly from liver glycogen; some glucose absorbed from the alimentary tract may serve directly in muscle glycogen synthesis. Major part of the glucose uptake from blood by skeletal muscle is dependent upon insulin (Beitz, 1993), which appears to be able to increase the uptake as much as five-fold at very high concentrations (Prior *et al.*, 1984). However, despite denial of food and water mature exhausted lambs (Chrystall *et al.*, 1981), and adrenalin-treated heifers (McVeigh and Tarrant, 1982) were able to replenish their muscle glycogen stores.

In sheep, glucose uptake by the muscle is greatest in well-fed, non-pregnant, non-lactating animals. The amount taken up could account for 30-40% of the oxygen consumption, assuming that it is all oxidized. By using ^{14}C-glucose, Lindsay (1981) found, however, that on average only 6% of CO_2 appeared to be derived from glucose. Possible explanation for this could be the synthesis of muscle glycogen, for which the glucose taken up could have primarily been used.

Table 1. Resting bovine muscle glycogen concentrations measured by various authors.

Muscle	[Glycogen] (mmol/kg)	Type of cattle	Reference
LTL	82–86	Simmental, Hereford, Angus	Crouse *et al.*, 1984
LTL	87	Irish Friesian bulls	Lacourt & Tarrant, 1985
LTL	58	French Friesian bulls	Lacourt & Tarrant, 1985
LTL	91–110	Beef breed x Friesian steers	Lambert *et al.*, 1998
LTL	90	Friesian bulls	McVeigh & Tarrant, 1981
LTL	80–111	Hereford heifers	McVeigh & Tarrant, 1982
LTL	90–94	Friesian bulls	McVeigh *et al.*, 1982
LTL	77, 80	Pirenaico, Brown Swiss bulls	Sanz *et al.*, 1996

Entire muscles can be classified to red/white, slow/fast, oxidative/glycolytic (Ashmore and Doerr, 1971; Peter *et al.*, 1972) according to the fiber types predominantly represented in the muscle. In well-fed and rested sheep, the glycogen level was markedly higher in the fast-red LTL and A (*adductor*) (90 to 105 mmol/kg) than in the fast-white (about 75 mmol/kg) or slow-red (about 80 mmol/kg) muscles. In bovine muscles, however, the differences may not be quite so marked, but a trend for a higher glycogen level in faster muscles could be observed (Monin, 1981; Lacourt and Tarrant, 1985).

Zerouala and Stickland (1991) compared muscle fiber type characteristics in normal versus dark-cutting beef carcasses. They found that there were proportionately more slow, oxidative (SO) fibers in dark-cutting (DC) bulls and steers than in normal bulls. DC bulls also contained fewer of the fast, glycolytic (FG) fibers than DC steers and normal bulls. Furthermore, when all oxidative muscle fibers (SO and FOG) were taken into account, the two dark-cutting groups, and particularly the DC bulls, exhibited significantly more oxidative metabolism in the LTL muscle than the normal animals.

Biochemical analysis of glycogen concentration

The results on glycogen concentration were found to be very similar if the hydrolysis of glycogen was done by incubation in acidic medium (HCl) or enzymatically by *amylo-α-1,4-α-1,6-glucosidase*, both followed by enzymatic analysis of the resulting glucose (Passoneau and Lauderdale, 1974). However, glucose is known to be slowly destroyed when heated in neutral solution and the rate of destruction is accelerated by sulphuric acid, but a much greater extent by hydrochloric acid (Desmecht *et al.*, 1995). On the other hand, with the commonly used method of homogenizing muscle into perchloric acid (PCA) and analyzing glycogen from the precipitate has been reported to underestimate the total concentration of glycogen, since only a portion of the total glycogen is extracted during PCA treatment (Hultman, 1967; Karlsson, 1971; Hermansen and Vaage, 1977). Jansson (1981) compared this method to those based on glycogen hydrolysis with acid or enzyme and documented that about 15-25% of the glycogen was PCA soluble.

Investigators studying glycogenin, the protein core of glycogen molecule, have recently found that there were two pools of glycogen in rodent muscle and other tissues such as liver and heart. One of the fractions was of smaller molecular weight (400,000) and relatively rich in protein,

and the other type was the "classic" glycogen of larger molecular weight (10,000,000) (Lomako *et al.*, 1991; Lomako *et al.*, 1993; Alonzo *et al.*, 1995). These molecular forms are referred to as proglycogen (PG) and macroglycogen (MG), and they differ in the ratio of protein to carbohydrate.

PG was found to precipitate in trichloroacetic acid because of its 10% protein component (Lomako *et al.*, 1993), while MG was soluble because of its low protein content of 0.35%. Adamo and Graham (1998) compared the traditional glycogen methods, with acid or enzymatic hydrolysis, to macroglycogen and proglycogen analysis of glycogen using both rodent and human muscle. They found that the PG fraction was always in excess of MG, which was 6-10% of total glycogen in rodent muscle. In human samples, however, the molar proportion of macroglycogen as glucose residues increased to about 40% when total glycogen was high. At a total glycogen concentration of 11 mmol/kg, the MG:PG ratio was 13:87. As the total glycogen increased to 46 mmol/kg the ratio increased to 19:81. At glycogen concentrations of 85 mmol/kg and 133 mmol/kg the ratios increased further to 25:75 and 38:62.

Adamo and Graham (1998) also documented that the methods based on acid and enzymatic hydrolysis of glycogen did not give systematically and significally different results, and that the determination of total glycogen as MG + PG did not differ from either of the two.

Utilization of glycogen

Many details of the fine structure of the glycogen molecule are still unknown, but the model of Whelan, mainly derived from data on enzymic degradation of glycogen is generally accepted (for details, see Meléndez-Hevia *et al.*, 1993). The degree of branching of glycogen molecule is equal to 2 so that in every B-chain, there are two branches creating new A- and B-chains (see Figure 1a.). There are four glucose residues between branches and a tail of four residues after the second branch in the B-chains. Every B-chain is, thus, in the inner tiers, whereas all the A-chains are within the outer tier (Figure 1b.).

It has been demonstrated by several groups that the availability of phosphorylated glucose (G-1-P) is the most critical variable in controlling glycolytic flux (cf. Meléndez-Hevia *et al.*, 1993). Furthermore, the limit for the action of *phosphorylase* is about 4 glucose residues (Walker and Whelan, 1960). From the above data it has been derived that there are the same number of A-chains (all of them in the last tier) as B-chains, and that the amount of glucose directly available to be released from glycogen molecule by *phosphorylase* is 34.6% of the total molecule, independent of the size (number of tiers) of the glycogen particle (Meléndez-Hevia *et al.*, 1993). Interestingly, Passonneau and Lauderdale (1974) analyzed the proportion of outer tiers of total glycogen concentration by digesting the sample with *phosphorylase A* but not with the debranching complex, and ended up with 32.2%. As it is also known that while *phosphorylase* is extremely abundant and active in skeletal muscle (Ryman and Whelan, 1971), the activity of glycogen debranching enzyme has, at the maximum, only 5–10% of the activity of *glycogen phosphorylase*. The weight ratio of *phosphorylase* to debranching enzyme is 10:1 in skeletal muscle, and this limited the rate of liberation of G-1-P from the *in vitro* substrate (limit dextrin) to 10% of the maximum speed (Taylor *et al.*, 1975).

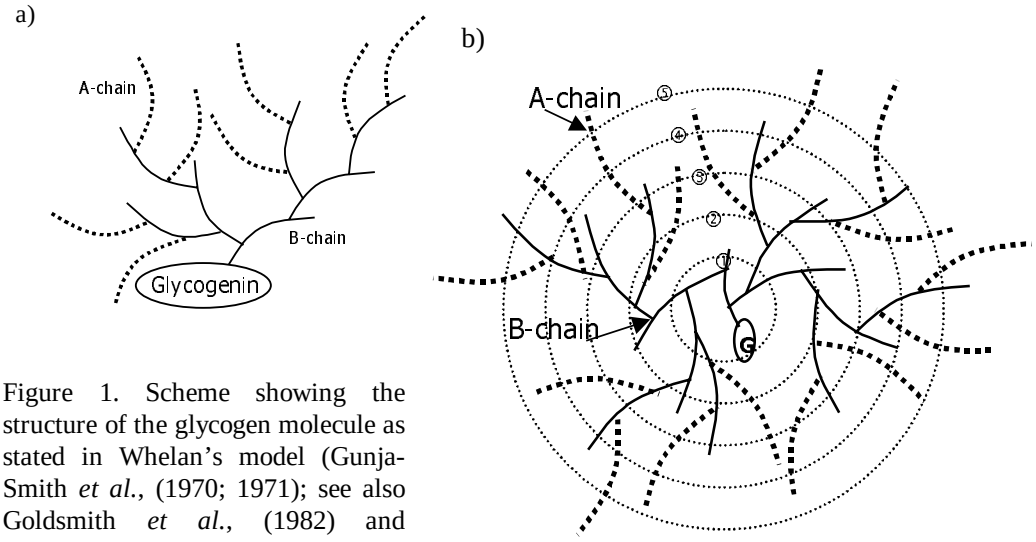

Figure 1. Scheme showing the structure of the glycogen molecule as stated in Whelan's model (Gunja-Smith *et al.*, (1970; 1971); see also Goldsmith *et al.*, (1982) and Meléndez-Hevia *et al.*, (1993).

The degree of branching (r) is 2; therefore, each tier has twice the number of chains as the previous one. Each A- or B-chain has 12-14 residues and there are 12 tiers (t=12) in the molecule, which means about 53 000 glucose residues. **a)** Extended structure to show the branching structure; **b)** a more realistic drawing showing the disposition of the successive branches forming concentric tier (numbered circles). Figure and the text are adapted from Meléndez-Hevia *et al.* (1993).

The key regulatory enzyme involved in the breakdown of muscle glycogen is *glycogen phosphorylase*, which can be either in active (*phosphorylase A*) or inactive (*phosphorylase B*) form. This phosphorylation of glycogen yields glucose-1-phosphate (G-1-P), which is then rapidly converted to G-6-P by *phosphoglucomutase*. This degradation begins from the non-reducing ends of each chain (A-chain) and continues until four glucose residues of a branch remains (B-chain). The branches of the glycogen molecule, on the other hand, are eliminated by a debranching enzyme complex of dual activity: first transferring (*transferase*) the chain of three glucose residues to another non-reducing end and then cleaving the last remaining residue at the α–1,6 linkage as free glucose (*amylo-1,6-glucosidase*) (Beitz, 1993).

Glycogen is mobilized by unaccustomed physical exercise and also by acute or chronic environmental stressors. The degradation of glycogen can be induced by adrenaline (hormonal) binding to the β–adrenoreceptors on the muscle cell membrane; by electricity (contractile) either by a natural action potential leading to muscle contraction, or exogenous electrical stimulation; or by a combination of adrenergic and contractile mechanisms. Also fasting, if continuing long enough, depletes glycogen slowly (for a concise review, see Tarrant (1989a)). Thus, although the stimulus to glycogen metabolism may differ – physical, emotional or environmental – the metabolic response will have a common basis (Lister, 1989). All cattle subjected to a given stressor do not react the same. Temperament and excitability are hereditary (Stricklin *et al.*, 1980) and variation in animal temperament is common.

It takes very little adrenaline to stimulate the initial enzyme, *adenyl cyclase*. Nanomolar concentrations of the hormone lead to micromolar concentrations of cAMP within the cell (Lehninger, 1993). In muscle, it has been estimated that a given concentration of cAMP initiates the formation of 25 000 times as much G-1-P per minute (Heffron, 1981). The effect of

adrenaline is to potentiate the activation caused by Ca^{2+} (Lehninger, 1993). This activation occurs at similar Ca^{2+} concentrations, which initiate muscle contraction (about 10^{-6}). The enzyme protease kinase has four binding sites for calcium ions, but is active already when only two calcium ions have been bound.

Glycogen is used as fuel for the exercise of skeletal muscle. The rate of use depends on work intensity (Pethick, 1993). Exercise-induced depletion of muscle glycogen does not occur readily in cattle. Howard and Lawrie (1956) found that 1.5 h of exercise were insufficient on its own to cause a significant reduction of glycogen levels in the LTL muscles of hard driven steers. Exercise combined with long train journey did, however, cause a slight elevation in ultimate pH (Howard and Lawrie, 1956). Fasting, on the other hand, lowered the glycogen level in LTL of steers but not sufficiently to affect ultimate pH. Dark-cutting was not produced by fasting 7, 14 or even 28 days (Howard and Lawrie, 1956). Lambert *et al.* (1998) walked steers over a 2.5 km course once or twice at a speed of either 4 km/h or 8 km/h and found that exercise did not affect glycogen concentration in the LTL muscle in any combination. The authors concluded that walking mature, well-fed steers of moderate live weight (400–500 kg) at a moderate speed for 40 minutes is not likely to significantly deplete muscle glycogen content. They speculated that fast-walking of steers does not elicit the same generalized physiological effect as fighting/mounting activity of bulls; and/or they did not exercise the steers strenuously enough, or for long enough to deplete glycogen; and/or release of adrenaline may be required before the level of exercise they imposed would significantly reduce muscle glycogen.

Glycogen utilization may be influenced by the concentration of glycogen. It has been suggested that preexercise glycogen concentration correlates positively with the rate of glycogen breakdown during exercise in man (Bergström *et al.*, 1967; Gollnick *et al.*, 1972; Galbo *et al.*, 1979; Gollnick *et al.*, 1981) and rats (Richter and Galbo, 1986).

However, it should be remembered that oxidative metabolism normally plays a dominant role in skeletal muscle ATP production. It is a well-established concept that oxidation of fat [mainly acetate and ketone bodies in ruminant muscles] can provide essentially all the energy required by working muscle during light to moderate exercise (Bechtel and Best, 1985). Furthermore, only working muscle groups utilized glycogen during the work period (Bergström and Hultman, 1966) and no glycogen depletion was measured in the resting muscle groups. The beef muscles with the highest pH values, ie., LTL, *semitendinosus*, *semimembranosus*, *adductor* and *gluteus medius*, may be selectively active prior to slaughter, as could be the case in repetitive strenuous activity such as aggression and mounting (Tarrant and Sherington, 1980).

McVeigh and Tarrant (1981) mixed bulls in pairs with an established group of bulls. One of each pair was given propranolol, a drug that blocks the β–adrenoreceptors of muscles and thus, prevents the effect of circulating adrenaline on glycogen metabolism. Muscle biopsy samples were taken 1, 3 and 5 h after initiation of the mixing during the stress and then again at 24 and 72 h during recovery. The mixing resulted in a high level of physical activity, which eventually led to exhaustion and fatigue in both control and propranolol-treated animals. Muscle glycogen concentration got lower at all sampling points during the stress in both treated and untreated bulls. Propranolol had a small protective effect against glycogen depletion during the initial part of the stress, but was finally totally ineffective in preventing glycogen loss, which after 5 h of stress increased to 63% and 55% in propranolol-treated and control bulls, respectively. The authors concluded that glycogen depletion during mixing stress is not predominantly mediated by catecholamines (McVeigh and Tarrant, 1981). The glycogen depletion during mixing is most probably caused by the increased intracellular energy demand during muscle contraction,

combined with β–adrenergic activation of glycogenolysis. Propranolol was effective, however, in reducing muscle glycogen depletion during a 4 h transportation stress in lambs (Monin and Gire, 1980), which indicates that, contrary to mixing stress in young bulls, glycogen breakdown during transportation may be predominantly mediated by catecholamines (Tarrant, 1989a,b).

In young bulls subjected to mixed penning, glycogen loss was greater in fast-twitch fibers than the slow-twitch fibers (Lacourt and Tarrant, 1985). On the contrary, adrenaline administration caused a greater loss of glycogen in the slow-twitch fibers, which may, thus, be more responsive to circulating adrenaline than the fast-twitch fibers (Lacourt and Tarrant, 1985).

Crouse and Smith (1986) found, that in biopsies taken 0.5 and 24 h after a 15-min period of continuous muscle contraction, glycogen content of LTL muscle was not different from precontraction concentrations. Subcutaneous administration of adrenaline resulted in a 30–35% reduction of muscle glycogen. Reduction was slightly greater when the two treatments were combined compared to the effect of adrenaline alone. The authors concluded that the data indicates that reports of increased glycogenolysis observed in cattle subjected to mixing stress is due to dynamic muscle contraction and not due to isometric muscle contraction.

In practice, stressful situations may have both physical and emotional effect in varying degree. An animal may experience emotional stress during transport followed by physical stress during mixed penning at the abattoir (Tarrant, 1989a,b). It has been suggested that the main cause of glycogen depletion and the occurrence of dark-cutting is the physical and emotional stress of mixing unfamiliar bulls overnight (Puolanne and Aalto, 1981; Sanz *et al.*, 1996).

The average rate of glycogen depletion is slower in ruminants compared to monogastrics. Whereas men during intensive exercise utilize glycogen at the rate of 64 µmol/g/h (Hultman *et al.*, 1974) and horses during high-speed trotting at the rate of 678 µmol/g/h (Lindholm & Saltin, 1974), the average rate of glycogen breakdown in young bulls severely stressed by co-mingling or adrenaline administration was reported to be 10–11 µmol/g/h (range 5 to 24) (McVeigh & Tarrant, 1983; Tarrant & Lacourt, 1984). Even though the maximum utilization rate of 24 µmol/g/h is sufficient to affect meat ultimate pH after only one hour of mixed penning (assuming an initial resting glycogen content of 80 µmol/g and an absolute requirement of about 57 µmol of glycogen/g of muscle to achieve an ultimate pH of 5.5 in beef LTL muscle), it takes little over 2 h to affect meat ultimate pH with the average depletion rate (Tarrant, 1989a) or even 4 h if the initial glycogen content is around 100 µmol/g. It seems, therefore, that even when the energy demand of ruminant muscles increases, as in heavy exercise or in stress, their muscles are not likely to mobilize glycogen as fast as those of monogastrics.

Dietary effects on glycogen resynthesis

Attempts have been made to increase the availability of glucose to ruminants, and thus, enhance muscle glycogen synthesis, by causing intentional changes in ruminal fermentation. This modification may include 1) increase in the proportion of concentrate feed (in order to increase the amount of volatile fatty acids (VFA) and particularly the proportion of the only glucogenic VFA, propionate), 2) feeding of urea supplements along with concentrates (to promote microbial protein synthesis), 3) administration of selective antibiotics, such as monensin (to reduce methane energy losses and to promote increased propionate production), 4) use of probiotics, such as yeast cultures (to reduce the negative associative effect of concentrates on cellulolysis), and 5) feeding of nutrients in a form that will largely protect them from fermentation without affecting enzymic degradation lower down the gastrointestinal tract (Leek, 1993).

After depletion of glycogen levels due to fasting, strenuous physical exercise or stress of some sort, the repletion of muscle glycogen concentration starts. Transported and exercised (exhausted) lambs were able to slowly replenish their muscle glycogen despite denial of food and water (Chrystall *et al*., 1981). The mean ultimate pH values after 17h and 24h of rest, ie., 5.85 and 5.71, were still higher than in the control groups (5.52 and 5.62) (Chrystall *et al*., 1981). Howard and Lawrie (1956) concluded that resistance of steers to fasting appeared to be much greater than that of rabbit and other non-ruminants. Fasting for 96 h reduced the muscle glycogen concentration of bulls from 77 mmol/kg to 50 mmol/kg (35%) (Crouse *et al.*, 1984). Muscle glycogen remained low until day 3 when it started to elevate at a rate of 3 mmol/kg/d. The authors concluded that attempting to attain a glycogen buffer by fasting and refeeding is not feasible.

Cows fed high energy diets (metabolizable energy 200 MJ) with 50% of diet fed as concentrates have higher baseline serum glucose and insulin concentrations, compared with cows fed lower energy diets (metabolizable energy 110 MJ) with 10% of diet fed as concentrates (Holtenius *et al.*, 1996). On the other hand, cows fed diets equal in energy and protein but with different ratios of concentrates and forages have similar serum glucose and insulin concentrations (Holtenius *et al.*, 1996). Andersson *et al.*, (2000) administered glucose intravenously in Holstein bulls to test their glucose tolerance and the insulin response curve, since an acute increase in blood glucose concentration is a stimulus for insulin secretion from the pancreas. Following the infusion, the blood glucose concentration increased in 30 min from the baseline value of 3.8 mmol/l (range 3.6–4.1) to 10.2 mmol/l (range 8.8–11.5). In 240 min the glucose concentration slowly decreased back to the values similar to the baselines (3.2–4.7 mmol/l). The carbohydrate metabolism of ruminants does not allow extra storage of glycogen to be achieved in muscle even by feeding a diet rich in carbohydrate. However, glucose availability (for energy purposes or storage) could be, at the very least, increased by feeding diets containing ground corn (Lister, 1989). The ruminal digestion of corn versus barley starch may be pertinent to a glycogen enhancement strategy. Up to 15-20% of the starch of ground corn escapes microbial digestion in the rumen (Lindsay, 1981) and is absorbed from the small intestine as glucose, which supplements gluconeogenic glucose and may further elevate muscle glycogen concentration.

McVeigh and Tarrant (1982) fed heifers barley or hay, or alternatively fasted for 9 days, to achieve high, low and zero dietary energy intakes. During the control period, when all experimental animals were fed either hay or barley, the hay-fed animals had a glycogen concentration of 80–90 mmol/kg, whereas the barley-fed animals had significantly more glycogen, ie., 108-111 mmol/kg. Adrenalin treatment reduced the glycogen concentration to 38%, 27% and 25% of the control values in the barley-fed, hay-fed or fasted heifers, respectively. The rate of glycogen repletion through days 1 to 7 was 7.6 mmol/kg/d in barley-fed, 6.1 mmol/kg/d in hay-fed and 1.5 mmol/kg/d in fasted animals, respectively. The rate of repletion was not significantly different between the two fed groups. Nevertheless, feeding the high-energy diet led to a higher resting glycogen concentration in the muscle allowing glycogen to be synthesized somewhat in excess to the pre-stress concentrations. Furthermore, blood glucose was higher in animals fed barley compared to the hay-fed or fasted animals. The authors concluded that resting muscle glycogen concentration of beef heifers as well as the glycogen repletion rate are influenced by the type of diet (high versus low energy intake), and that the slow repletion rate in beef muscle was caused by a low glucose availability. They also noted that the fasting for 9 days lowered the rate of glycogen recovery to such an extent that is highly unlikely that a fasted, glycogen-depleted animal could recover sufficient glycogen after transportation to slaughter to ensure normal meat quality.

After mixing stress, the glycogen concentration of bulls increased at a rate of 6.6 mmol/kg/d for the first 7 days when feeding a concentrate barley diet (McVeigh *et al.*, 1982). For the first 2 days the rate was higher, ie., 14 mmol/kg/day. Muscle glycogen repletion rates reported in humans and rats are commonly at least 10 times higher than those of young bulls (cf. McVeigh and Tarrant, 1982). The authors concluded that slow repletion rates may be characteristic to ruminants, since Monin (1981) reported similar repletion values (19 mmol/kg/d) in sheep that had received adrenaline.

Miller *et al.* (1987) fed bulls either a high-energy diet designed to accelerate growth or a low-energy diet to defer growth. The accelerated dietary regimen contained 10.9 MJ of metabolizable energy (ME) per kgDM fed for the first 100 days followed by a finishing diet of 12.7 MJ ME/kgDM for the last 110 days. The deferred dietary regimen consisted of good quality pasture for the first 110 days, followed by finishing for 180 days on a high-energy dietary regimen of 12.7 MJ ME/kgDM. They found that bulls fed at the accelerated rate of growth had higher *postmortem* pH, and lower muscle glycogen stores than the bulls on deferred diet. The authors concluded that the increase of glycogen seems to be age-dependent, since the animals on deferred diet were 90 days older than the animals on accelerated diet when slaughtered, or perhaps it was the combination of age-dependence and longer feeding time rather than the different energy levels of the diets, they reasoned, that was responsible for the increase in muscle glycogen.

Pethick and Rowe (1996) investigated the effects of feed intake and exercise training on the level of glycogen in the muscles of 12-month-old Merino wethers. The diet was a pelleted ration consisting of cereal straw (20%), barley grain (53%) and lupin grain (26%), and provided 11.9 MJ ME per kgDM. The authors found that increased feed intake significantly increased glycogen levels in both muscle groups (*M. semimembranosus* (SM) and *M. semitendinosus* (ST)) at both sampling times (week 6 when the sheep had received 2 weeks of exercise training, and at 48 h *postmortem*). There was a strong linear relationship between feed intake and glycogen level in sheep fed 1, 1.3, 1.5, or 2.2 times their maintenance energy requirement. The increasing level of feed intake also significantly reduced the ultimate pH values of all muscle groups, with the effects being most pronounced in the ST.

In a study on the effect of diet and exercise on bovine glycogen levels, Pethick *et al.* (1994) found that the increase in ME intake was a major determinant of increased muscle glycogen concentration in barley supplemented cattle that had received 8 exercise sessions. After five more training sessions, the effect of nutrition was not significant anymore, since the effect of training exercise became more important on the increasing glycogen content than nutrition.

Vestergaard *et al.* (2000) studied the effect of two production systems on various characteristics of Friesian bulls in Denmark. In the extensive (Ext) system, animals were loose-housed and fed a roughage-based diet from October to May, followed by a grazing period from May to October. A group of Ext-bulls (at mean live weight of 360 kg) was slaughtered directly from the pasture and the rest were finished in tie-stalls for 10 more weeks with concentrates *ad libitum*, and slaughtered at mean live weight of 460 kg. In the intensive (Int) system, animals were tie-stall housed and *ad libitum* fed until slaughtered at live weights of 360 and 460 kg. The Ext-bulls had lower glycogen content than the Int-bulls at 360 kg, but the situation was reversed by 460 kg following the finishing feeding. The proportionate amount of SO and FOG fibers in the ST and LTL muscles was larger in Ext-bulls compared with Int-bulls, and the amount of FG fibers was lower in all three muscles (ST, LTL and *supraspinatus* (SS)) of Ext- compared to Int-bulls.

Wajda *et al.* (1982) provided bulls with molasses at a ration of 3 kg/d for the 3 or 6 last days of finishing. They found that feeding the molasses improved the quality of meat in terms of increased glycogen content, decreased ultimate pH, and increased lightness of fresh meat color. Lengthening the feeding period to 6 days did not improve the quality any further.

Wiklund *et al.* (1996) studied reindeer in relation to supplementary feeding (10 MJ ME /kgDM) and transportation prior to slaughter. Animals that received supplementary feeding for 2 months had average glycogen concentrations of 52, 68 and 47 mmol/kg across muscles (converted from dry weight concentration using the approximation that muscle contains 75% water), if they were not transported, transported for 500 km and slaughtered immediately, or transported and laired for 2 days with access to hay and water, respectively. The comparable concentrations across muscles for animals with no supplementary feeding were 25, 33 and 28 mmol/kg. Furthermore, reindeer that had been receiving 5 months of the supplementary feed and were slaughtered at the university facility with no pre-slaughter stress had average glycogen concentrations of 117, 126 and 87 mmol/kg in LTL, *biceps femoris* and *triceps brachii* muscles, respectively.

Yambayamba *et al.* (1996) subjected heifers to two dietary treatments. The ADLIB group was fed to appetite on a barley-based diet providing for 13.8 MJ ME/kgDM. The REST group was switched to maintenance ration at the beginning of the experiment (period 1), and back to *ad libitum* feeding on day 92 of the experiment (period 2). Some of the heifers at REST group were slaughtered after period 1. Muscle samples were obtained within 5 min following exanguination. There were no significant differences in the concentration of glycogen or its metabolites in the LTL (LL) muscle of REST compared with ADLIB heifers at the end of period 2. However, heifers slaughtered after period 1 had lower glycogen concentration (55 mmol/kg) than heifers slaughtered after period 2 (77 mmol/kg).

Recent results of Daly *et al.* (1999) offer an interesting insight into the control of muscle glycogen concentrations in cattle. They finished Angus steers (previously grown together on pasture) for the last nine weeks either on a good quality spring pasture (ryegrass/clover) or on a grain-based feedlot ration (corn, heat-treated soy, and hay). The provision of feed was restricted on the feedlot-finished steers in order to achieve comparable growth rates as well as prohibit the confounding effect of energy intake on the potential effect of the type of feed. The carbohydrate concentration was over five times higher in grain diet than in the pasture diet. While the attempt of producing cattle of equal weights was unsuccessful and the grain-fed cattle ended up with lower weight gains, slaughter weights, and body fat contents, the glycolytic potential as an estimate of pre-slaughter glycogen content was yet approximately 20% higher in the grain-fed steers compared to the pasture-fed steers, the residual glycogen content as much as five-fold in the grain-fed compared to the pasture-fed cattle. Ultimate pH values were low for both groups. Although the authors finally concluded that perhaps grain-based diets can increase glycogen concentration independent of the higher calorific intake normally associated with grain diets, probably in response to diet-induced alterations in rumen fatty-acid production, they did not exclude the possibility of greater glycogen depletion due to pre-slaughter handling in the pasture-fed steers from a supposedly equal on-farm concentration. After all, there was an 18-hour feed withdrawal, 30-min transportation, overnight lairage at the abattoir facility, slaughter itself, and 48-hour refrigeration for the animals on either diet before the muscle samples were obtained.

It seems, therefore, that in bovine animals the ruminal fermentation, the availability of gluconeogenic precursors for hepatic glucose production, the availability of glucose for potential elevation of blood glucose, as well as the concentration of muscle glycogen are at least to certain extent responsive to changes in dietary regimen.

Formation of ultimate pH

Buffering capacity

The relation of pH fall *postmortem* to lactic acid (LA) formation is conventionally expressed as the buffering capacity, BC, which is the slope of the curve relating the two parameters (BC = –dLA/dpH/g). In most of the muscles of mammals, BC is constant from about pH 7.0 down to the final pH (5.4-5.8) (Bendall, 1979).

BC is an ability of weak acids to resist the change of pH when acid or alkali is added. Muscle fibres have this characteristic of weak acids mainly because they contain phosphate compounds and compounds carrying an imidazole group (Kivikari, 1996). After slaughter, BC of the muscle fibres determine the molar concentration of lactic acid (LA) needed to lower the pH of one kg of muscle by one pH unit. The buffering capacity of bovine muscles has been studied by several authors (for an excellent review, see Kivikari, 1996), and the arithmetic mean of their results is 57 mmol LA /(pH kg). According to Kivikari (1996), the mean BC of bovine LTL is 51 mmol LA /(pH kg). Because every mole of glucose yields two moles of lactic acid, the amount of glycogen needed for the maximum pH fall from 7.2 to 5.5 would then be about 43 mmol/kg expressed as glucose.

Already Howard and Lawrie (1956) pointed out that there may be appreciable differences in the buffering capacity of bovine muscles from the mean of 49 LA eq./kg pH. Variations above or below the standard value would tend to shift points on the 'initial glycogen-ultimate pH curve' to right or left respectively (Howard and Lawrie, 1956). According to Bate-Smith (1938), muscles can differ in buffering capacity both quantitatively and in the substances contributing to it. Furthermore, BC is higher in muscles considered predominantly white compared to red ones (Davey, 1960; Kivikari, 1996).

About 100 mmol/kg lactate is produced in muscle having a normally low ultimate pH (5.5) whereas only 40 mmol/kg lactate would be expected in a dark-cutting muscle with an ultimate pH 6.2 (Davey and Gilbert, 1976).

Glycogen concentration at slaughter

Howard and Lawrie (1956) studied the relationship between initial glycogen content of beef muscle and its ultimate pH of 5.44. They found that when a pH of 5.44 is attained there are generally considerable reserves of glycogen in beef *psoas major* (PM) and LTL muscles. From the mean ultimate pH of 5.44 and the slope (Δglycogen/ΔpH) of 0.396, the minimum quantity of glycogen necessary to attain this pH level, from the initial 7.2, is about 700 mg/100 g. Taking the authors' error margins into account this equals to 34–45 mmol/kg. The highest initial glycogen concentrations measured by Howard and Lawrie (1956) were around 135 mmol/kg, and the concentrations between 39 to 84 mmol/kg giving the low ultimate pH around 5.44 to 5.5 were well represented.

McVeigh (1980 as reported by Tarrant, 1989b) developed a model to describe the dependence of ultimate pH on the pre-slaughter glycogen concentration in bovine LTL muscle. Based on his results, 57 mmol/kg was sufficient to lower the pH of beef from 7.1 to 5.5.

Warriss (1990) presented a figure on the relationship of ultimate pH to the concentration of glycogen present in the LTL muscle at death. His data on the curvelinear dependence consisted of 2345 observations and revealed that pH fall appears to be limited only approximately below the glycogen concentration of 45 mmol/kg. It can be noted from the figure that the lowest and highest glycogen concentrations of his data were approximately 8 and 104 mmol/kg, respectively. Similarly, Fernandez and Gueblez (1992) obtained the best prediction of the relationship between porcine glycolytic potential (GP) (an estimate of pre-slaughter glycogen concentration expressed as lactate equivalents as proposed by Monin and Sellier (1985): [GP] = [lactate] + 2([glycogen] + [glucose-6-phosphate] + [glucose])) and ultimate pH using a segmented quadratic model with plateau. They concluded that pH_u decreases following a curvelinear regression when GP increases until the convergence point (GP=173mmol/kg). Above this threshold, pH remains at a constant value (5.50) regardless of GP.

Various authors have reported glycogen concentrations at the time of slaughter and the corresponding ultimate pH values. Lahucky *et al.* (1998) measured glycogen concentration immediately prior to slaughter from control and stressed bulls. The glycogen concentrations were 61 and 33 mmol/kg, respectively, producing ultimate pH values of 5.66 and 6.70. Miller *et al.* (1987), on the other hand, took LTL samples immediately after exanguination (0 h) from control bull carcass sides and sides subjected to electrical stimulation within 1 h *postmortem*. The glycogen concentrations were 55 and 41 mmol/kg at 0 h in the control sides and the sides to-be-electrically stimulated, whereas after 2 h and thus, after electrical stimulation, the glycogen concentration had decreased to 35 and 21 mmol/kg ie., 36 and 49% of the initial values. Bidner *et al.* (1981) fed steers four diets varying in the type of pasture and in the amount of grain/concentrate supplemented with. The diets had no influence on the ultimate pH of meat. The facts about duration of transportation or holding time before slaughter were not given!

Residual glycogen concentration

Anaerobic glycolysis of bovine muscles ceases, when pH has reached 5.6 ... 5.3 (Howard and Lawrie, 1956; Greaser, 1986), even in the presence of large amounts of residual glycogen (Lawrie, 1955; Bendall, 1973). The reasons for this phenomenon are not clearly understood. According to Sahlin (1978), glycolytic enzymes are inactivated when pH reaches low values (<5.4). Consequently, the dependence of pH on the glycogen concentration of the time of slaughter is not linear, as has been demonstrated by several authors (Howard and Lawrie, 1956; Warriss *et al.*, 1984; Warriss, 1990; Fernandez and Gueblez, 1992; Przybylski *et al.*, 1993). Przybylski *et al.* (1993) concluded that pH decreases following a curvelinear regression when glycolytic potential increases, until a plateau value dependent on the animal species and muscle.

Bousset (1982) formulated the dependence of residual glycogen on ultimate pH to an equation; pHu=7.0-log(glucose mg/100g). However, the data was based on few animals only, and the residual glycogen values were quite low, from the approximate minimum of 0.5 mmol/kg to the approximate maximum of 20 mmol/kg. Furhermore, normal low ultimate pH values of 5.4–5.5 were not observed, the majority of data points represented pH range from 5.7 to 6.2.

Residual glycogen concentrations have been reported by many authors, but they mainly concern pork. Aalhus *et al.* (1998) compared the characteristics of pale, soft and exudative (PSE) beef and pork. The determination of bovine PSE was based on a subjective rating carried out by an experienced research technician and meat grader. The residual glycogen concentrations were similar in normal and PSE beef, about 35–40 mmol/kg. Lahucky *et al.* (1998) presented residual

glycogen concentrations that were equally low in control bulls of low pH 5.66 (17.4 mmol/kg) and in stressed bulls of high pH 6.70 (18.3 mmol/kg).

Residual glycogen concentrations of pork are somewhat lower in average than those of beef, but the high concentrations reported in pork at comparable level with beef. Seewer *et al.* (1996) studied some metabolic characteristics of LTL samples of specific pH_{24} values in Swiss Large white pigs. The mean residual glycogen-glucose concentrations for different pH groups were 12.5 mmol/kg (pH≤5.36), 16.2 mmol/kg (pH 5.37–5.55), and 6.4 mmol/kg (pH≥5.56). The variation in glycogen level, from approximately 7 to 34 mmol/kg, was greatest at pH 5.37–5.55, whereas it was from 4 to 20 mmol/kg at pH≤5.36 and from 3 to 13 mmol/kg at pH≥5.56. In the study of Maribo *et al.* (1999), the mean residual glycogen concentrations were about 8 mmol/kg in porcine LTL muscle. The minimum and maximum concentrations observed were 0 and 61 mmol/kg.

Melton *et al.* (1982) studied the flavor and chemical characteristics of ground beef from steers finished with grass, forage-grain or grain either during winter or summer. The mean residual carbohydrate (glycogen + glucose) concentration in the ground beef varied between 34–44 mmol/kg. Ground beef from grass-fed steers slaughtered in summer had a lower carbohydrate content (34 mmol/kg) than ground beef from limited grain-fed (41 mmol/kg) or grain-fed (43 mmol/kg) steers slaughtered in summer as well as in ground beef from silage and limited grain-fed (44 mmol/kg) or grain-fed (44 mmol/kg) steers slaughtered in winter.

Pethick and Rowe (1996) reported of residual glycogen contents of 17–34 and 35–51 mmol/kg in SM, 17–34 and 28–45 mmol/kg in LTL and 0–10 and 7–17 mmol/kg in ST muscles of sedentary and regularly exercised sheep, respectively. They also noted that in meat ultimately having a pH of 5.6, glycogen concentration decreased 20 to 110 mmol/kg during the post-slaughter period of 48 h. However, consumed concentrations above 50 mmol/kg *postmortem* are somewhat in disagreement with the concept of buffering capacity.

Residual glycogen-glucose effects on the physical and sensory quality of beef

Monin *et al.* (1987) hypothesized that the level of residual glycogen could influence the technological yield of cooked ham processing independently of its effect on ultimate pH. They suggested that the water bound by glycogen molecule is likely to be freed during *postmortem* glycogenolysis as well as during the processing of the meat, and that this could lead to an excess of free water relative to the protein network susceptible to hold it, thus inducing and extra release of water during cooking. Glycogen concentration is indeed a potential contributor to the water holding capacity of meat, because glycogen molecule binds 2 to 4 times its weight's worth of water (Olsson and Saltin, 1970).

In a review on glycogen metabolism and meat quality, Pethick *et al.* (1995) speculated with the many potential and positive effects of residual glycogen concentration on meat quality: "Firstly, glycogen allows for improved keeping qualities since the microbial population uses glycogen as a fuel rather than protein. Utilization of protein by bacteria results in the production of ammonia and "off" odors and flavors (Gill and Newton, 1981). Secondly, glycogen is a very hydrophilic (water loving) molecule (3–4 g water/g glycogen; Olsson and Saltin, 1970) and so contributes to the moisture content of meat. Meat with a high pH_u and so low residual glycogen is not only dark in color but also dry in texture (i.e. DFD). Indeed a loss of glycogen preslaughter implies a

significant loss of carcass weight. Finally residual glycogen is thought to undergo browning reactions with protein during the cooking process and so further contribute to flavor – however the latter has not yet been scientifically tested".

Boers *et al.* (1994) suspected that the poor shelf life of pork compared to that of beef could be due to differences in residual carbohydrate concentration of the meat. To test this, they studied the shelf life of vacuum-packaged wild boar meat in relation to that of vacuum-packaged pork. Wild boar meat did, indeed, prove to have a much longer storage life than pork and resembled that of beef. The authors were surprised of the very high glucose content of the wild boar meat (2500–6000 μg/g) compared with average values of 1000 μg/g reported for pork (Fischer and Augustini, 1977; Gill and Newton, 1981; Kress-Rogers *et al.*, 1988). The authors speculated that the reason for this could be a quick conversion of glycogen into glucose as a result of hunting stress, and thought that the fact that there were two loins, in which no glycogen could be detected at all, supports this view. They concluded that the LTL muscle of wild boars is rich in glucose, and this may contribute to its long shelf life through a delay in glucose limitation and subsequent amino acid breakdown by microorganisms. They also suggest that the onset of spoilage strongly depends on the glycogen/glucose evolution of the meat.

Grain-feeding of steers have been found to result in increased free sugar content (Brown et al., 1979), increased tenderness, more desirable flavor and increased overall palatability of beef than forage feeding of steers (Bowling *et al.*, 1977; Dikeman *et al.*, 1985). In addition, grain-finished beef had twice as much subcutaneous fat as forage-finished beef, the longest sarcomeres and the lowest shear force values (Bowling *et al.*, 1977). In the study of Brown *et al.* (1979), the content of free sugars increased significantly during frozen storage (-34 °C) of 30 to 180 days. The authors did not speculate as to whether this increase was due to the hydrolysis of glycogen present in the meat. The free sugar concentrations were 64 and 147 μg/g in low-energy-grass-fed, 81 and 175 μg/g in low-energy-grain-fed, and 78 and 162 μg/g in high-energy-grain-fed beef after 30 and 180 days of storage, respectively. Melton *et al.* (1982) found that the ground beef from steers finished in winter had the highest free sugar content and flavor scores. A desirable flavor in beef has been associated of higher intensities of a browned flavor (Berry *et al.*, 1980; Melton *et al.*, 1982). Contrary to these results, Bidner *et al.* (1981) observed that beef from steers finished on all-forage, forage-plus-grain or high energy did not differ from each other according to color of fat, Warner-Bratzler shear force values, or consumer evaluation of steaks for tenderness, juiciness, flavor and overall desirability. The color of lean muscle was darker, however, from forage cattle as measured by visual scores and by the Hunter Color Difference meter. Residual carbohydrate concentrations were not analyzed.

The supposed tenderness of animals fed on a high-energy diet could also relate to the fact that such animals would have high muscle glycogen levels (Lister, 1989) compared with other animals, allowing them to maintain a lower ultimate pH in the face of stressors associated with slaughter (Devine *et al.*, 1993).

During the early days of *postmortem* the concentration of free glucose rises on the meat surface (Kress-Rogers *et al.*, 1993), supposedly because of hydrolysis and diffusion of residual glycogen (Kress-Rogers *et al.*, 1993) and other fermentable substrates (Gill, 1976) from the deeper layers of the meat. It is also known that a wide variety of microorganisms, mainly molds, bacilli and some other gram-positive bacteria produce and excrete exocellular starch depolymerizing enzymes (reviewed by Fogarty and Kelly, 1979; 1990; Vihinen and Mäntsälä, 1989), which are capable of rapid impairment of the technical and hygienic quality of starch (Maher and Cremer, 1987). As a polymerized carbohydrate, the glycogen left over from the *postmortem* reaction

sequence of meat is most probably hydrolyzed to glucose during storage. All the answers are still not available. Are certain common bacteria perhaps capable of hydrolyzing glycogen efficiently in muscle? And if so, what enzymes are involved? To what extent could this microorganism-driven depolymerization of residual glycogen take place? How far does it continue? And does ground meat perhaps differ from a larger piece in this respect?

OBJECTIVES OF THE PRESENT STUDY

The ultimate goal of this work was to gain additional knowledge and tools for integrated beef quality control by attempting to maximize pre- and post-transportational glycogen reserves in order to secure the production of normal pH beef. Schematic illustrations present the relationship between the true variables (intentional, unintentional, treated, as well as varied) existing in the study (Figure 2.) and the concrete objectives related to the variables (Figure 3.).

The objectives of the present study were to investigate:

1. The effects and possible interactions of dietary energy density and male status on muscle glycogen concentration using the same set of animals for crossover dietary experiments (II).

2. The relationship between ultimate pH and residual glycogen-glucose concentration in bovine muscles (III).

3. The effects of a high-energy diet provided for a rather short time prior to transportation to slaughter on muscle glycogen concentration on the farm, after transportation, and after refrigeration of the carcasses (IV).

4. The effects of residual glycogen-glucose concentration on the physical and sensory quality of normal pH beef (V).

5. The variation of glycogen concentration according to biopsy location within bovine *longissimus thoracis et lumborum* muscle (I).

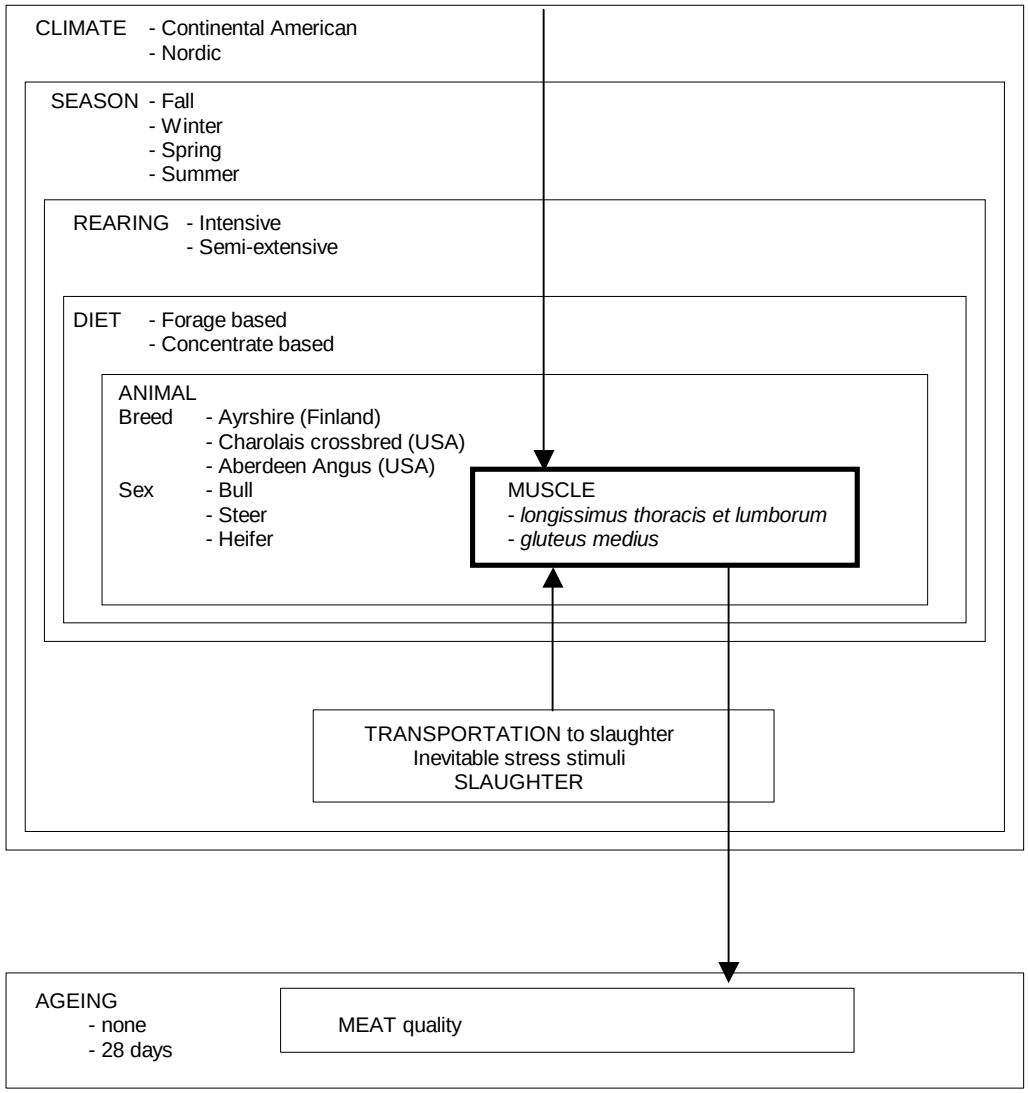

Figure 2. The set of variables: *true variables*

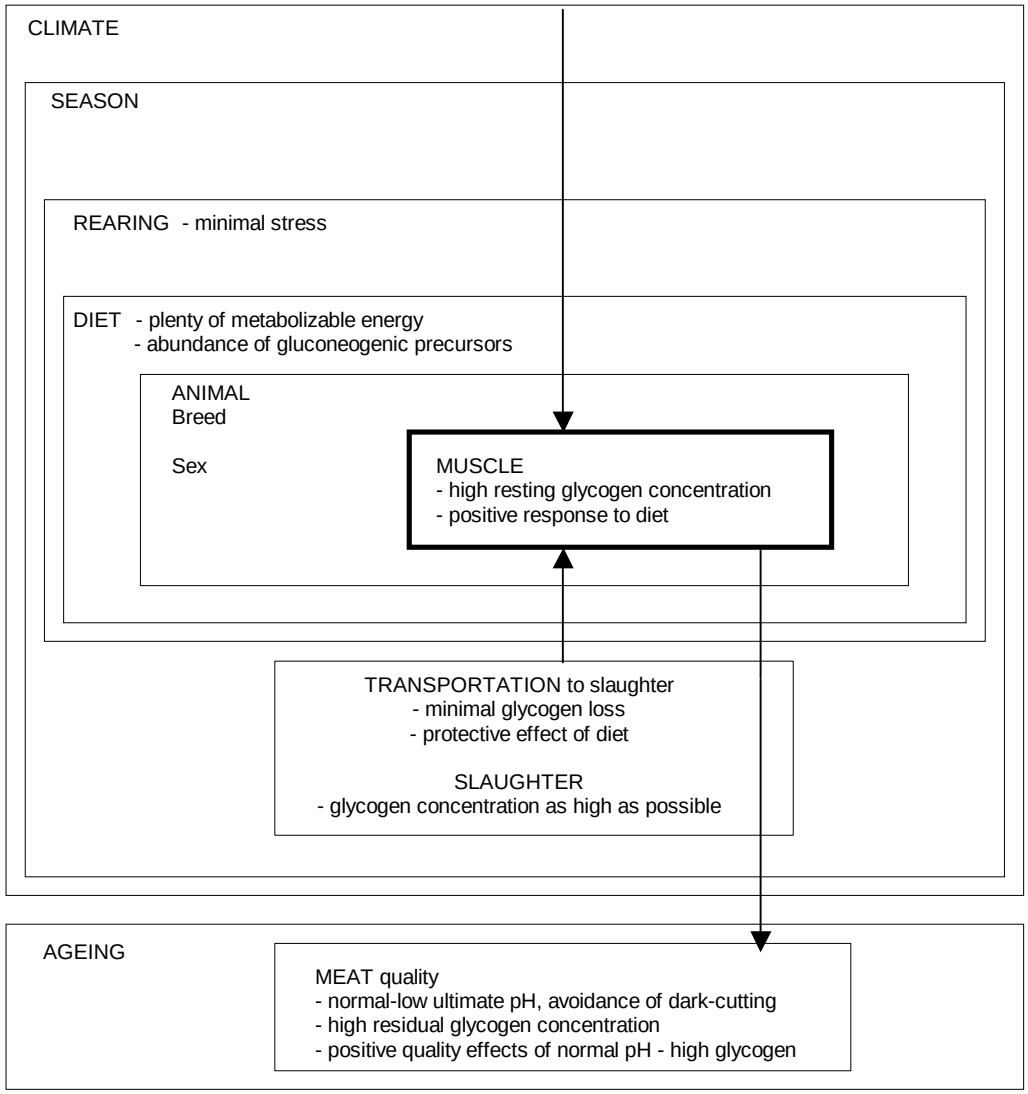

Figure 3. The set of variables: *objectives associated with the true variables*

MATERIALS AND METHODS

The materials and methods used in this work are described in detail in the original papers I–V.

Animals and diets

A total of 184 bovine animals were used in studies I–V. Of these 133 were Ayrshire bulls in Finland (III–V), 19 Charolais crossbred bulls (II), 25 Charolais crossbred steers (I–II), and 7 Angus heifers (I) in the U. S.

Ethical permissions to conduct the experiments were issued by the Research Animal Resources Committee of the University of Wisconsin, Madison (I–II) and local Research Animal Committee with the Provincial Government in Finland (IV).

Animals were housed in individual pens of 3 m^2 (I), in pens of 35 m^2, each accomodating 4 or 5 animals (II) (University of Wisconsin, Beef Nutrition Unit, Arlington, Wisconsin, USA) or individually tied in stalls (IV) (Sugarbeet Research Center, Perniö, Finland).

The diets in the experiments were:
1. 100 % alfalfa haylage (diet AH) (II)
2. 90 % (DM basis) corn and supplement : 10 % alfalfa haylage (diet C) (II)
3. 100 % grass silage (Low energy diet) (IV)
4. Compound feed and grass silage (High energy diet) (IV)

The compound feed consisted of 39% steam rolled barley, 39% molassed sugarbeet pulp, 15% rapeseed expeller, 3% molasses, 2.2% mineral-trace element-vitamin supplement, and 1.8% rapeseed oil.

All diets were offered *ad libitum.*

The energy contents (MJ metabolizable energy/kg dry matter) of the feeds were 9.5 (1), 13.2 (2), 10.8 (3) and 12.9 (4).

For study II (Wisconsin, USA), a protocol for gentle handling was adopted (Grandin, 1996, personal communication) and followed strictly for all experiments of that study (paper II). Cattle were trained to enter the restraint chute where muscle biopsies were eventually to be collected. Prior to the first biopsy session, this training took place 3–4 times per week for four weeks. Animals were allowed time to voluntarily enter the chute to eat very palatable feed offered to them. The enticement provided to the cattle was a grain mixture consisting of (dry matter basis, %) 44.5 whole oats, 34.8 cracked corn, 7 whole roasted soybeans, 3.6 dehydrated alfalfa pellets, 8.3 liquid cane molasses and 1.8 salt-mineral-vitamin-lasalocid supplement. When animals had learned to willingly enter the chute, restraining was gradually applied at first without but later with brushing and clipping of the hair at the eventual incision sites. Clipping was accompanied by firm touching. The training continued with diminishing frequency throughout all experiments (II).

Experimental design and sampling

An overview to the experimental design as well as muscle sampling and analysis in the studies I–V are presented in Tables 2 and 3, respectively.

Muscle biopsies were obtained from *longissimus thoracis et lumborum* (LTL) and *gluteus medius* (GM) muscles of with a Bergström needle (inner diameter 6 mm) under local anesthesia (Lidocain 20 mg/ml, 3 ml per site) as described by Lindholm and Piehl (1974). Prior to driving an animal onto the restrainer (IV), an injection of tranquilizer (Xylazin 20 mg/ml (Rompun®)) at a dosage of 0.30 ml/100 kg was given intramuscularly.

Animals were weighed at the time of sampling and their temperament was observed. In Wisconsin, the behavior of the animals was rated on a 4-point scale adopted from Grandin (1994) at the following times: 1) when entering the chute, 2) between anesthetization and sampling (5 min), 3) at the time of sampling, and 4) after sampling. In Finland, the behavior of the bulls during sampling was rated subjectively with a three-point scale designed for the present experiment (0=calm, 1=slightly restless, 2=totally beserk (=very aggressive).

Table 2. Overview to the experimental design in the studies I-V: Treatments.

Study	Experiment	Number of animals	Treatment group	Treatment
I	1	7	p.m. sampling	six per LTL at interval of 6–8 cm
	2	6	biopsy sampling	eight per LTL at interval of 6 cm
II	1	38	Diet crossover	30d AH[1] – 30d C[2] 30d C – 30d AH
			Male status	Castrated, Intact
	2	38	Diet crossover	(adr inj.) – 37d AH – 37d C (adr inj.) – 37d C – 37d AH
			Male status	Castrated, Intact
	3	38	Diet crossover	(adr inj.) – 30d AH – 30d C – slaughter (adr inj.) – 30d C – 30d AH – slaughter
			Male status	Castrated, Intact
III		133	N/A[3]	
IV		60	Diet	High energy, Low energy
			Season	Spring, Summer
V		42	Residual glycogen	≤ 25 mmol/kg 25.1 – 49.9 mmol/kg ≥ 50 mmol/kg
			Ageing	None, 28 d

[1] diet alfalfa haylage (100%)
[2] diet corn (90%)
[3] Not applicaple

Table 3. Overview to the experimental design in the studies I-V: Sampling and analyses.

Study	Experiment	Sampling time	Sampling location	Analyses
I	1	0–15 min after bleed		glycogen[1)], lactate
	2	0–15 min after anesthetic		glycogen[1)], lactate
II	1	prior to	LTL/12th rib, GM	glycogen[1)], lactate
		30d, at crossover	LTL/11th rib, GM	glycogen[1)], lactate
		60d	LTL/10th rib, GM	glycogen[1)], lactate
	2	24h post adrenaline	LTL/12th rib, GM	glycogen[1)], lactate
		37d, at crossover	LTL/11th rib, GM	glycogen[1)], lactate
		74d	LTL/1st sacral vertebra, GM	glycogen[1)], lactate
	3	48 h *postmortem*	LTL/12th rib, GM, SM	pH, glycogen[1)], lactate
III		40-48h *postmortem*	LTL/12th rib, GM, SM	pH, glycogen[1)], lactate
IV		prior to exp. diet	right: LTL/12th rib, GM	glycogen[1)], lactate
		after 14d on diet	left: LTL/12th rib, GM	glycogen[1)], lactate
V		40–48h *postmortem*	LTL/12th rib	pH, glycogen[1)], lactate
		prior to storage	LTL, various portions	fresh meat color drip loss
		after storage	LTL, various portions	thawing loss shear force frying loss color of fried steaks sensory evaluation

[1)] glycogen + glucose

Transportation to slaughter

Cattle were road transported to commercial slaughter houses with trucks. The transportation took 5–5.5 h. In the USA, fighting among intact males was not prevented during loading of the truck (about 1 h), transportation, unloading and lairage prior to kill (about 1.5 h). In Finland, stress was kept to minimum at all stages, and fighting of bulls was prevented by driving each animal individually to the truck, by confining 2–4 animals tightly into a compartment, and by lairing the bulls in individual pens while in the abattoir (less than 1 h).

Biochemical methods

Each biopsy was assayed for glycogen-glucose and lactate concentrations. For glycogen and lactate determinations, muscle samples were homogenized in ice-cold phosphate buffer (pH 7.0) with a Polytron homogenizer (I–II) or a Tissuetech homogenizer with a teflon pestle (III–V).

Glycogen-glucose

Ten µl of homogenate were hydrolyzed in 200 µl of 0.1 M HCl at 100 °C for 2 h, after which pH was adjusted to 6.5–7.5 (Lowry and Passoneau, 1973) and glucose was determined via $NADP^+$ reduction with a linked assay involving *hexokinase* and *glucose-6-phosphate dehydrogenase* (Glucose (HK) 16-50, Sigma Diagnostics).

Lactic acid

Lactate concentration was determined from the homogenate via NAD^+ reduction with a linked assay involving *lactate dehydrogenase* and *glutamate pyruvate transaminase* (Boehringer-Mannheim no. 139 084).

Quality control of the assays

QC routines and procedures

Muscle reference standards were included in all glycogen sample analysis sessions. Reference standards consisted of bovine *semimembranosus* (SM) and ovine *psoas major* (PM). Samples of these muscles were obtained soon after dehiding, frozen, ground in liquid nitrogen and stored at –80°C until aliquots were needed. At each analysis session, a small amount of either SM or PM was homogenized and assayed for glycogen along with other samples as described above.

The repeatability of the lactate assay was determined two times over the course of the experiment using the muscle samples described above, ie., SM (n=20) and PM (n=20). A recovery test using a stabilized known concentration of lactic acid provided by the manufacturer of the kit was performed at every analysis session.

Calculation of the glycogen concentration was based on a standard curve. The standards were prepared, tested for accuracy, divided into portions, frozen, held at –80°C, and used at each analysis session.

The mean glycogen concentration of the bovine SM was 77.9 ± 7.9 (sd) mmol/kg (coefficient of variation (CV)=10.1%) and the ovine PM 38.2 ± 3.2 mmol/kg (CV=8.4%). The lactic acid concentrations (n=20) were 21.6 ± 1.2 mmol/kg (CV=5.6%) and 36.8 ± 2.4 mmol/kg (CV=6.6%), respectively. The mean recovery of lactic acid was 98.8 ± 2.7%.

Laboratory intercalibration

Since studies of this work were conducted in two places and thus, biochemical analyses were performed in two laboratories by two persons, a laboratory intercalibration was necessary. For this purpose, a bovine sample was prepared as described above, and analyzed for glycogen-glucose and lactate concentrations ten times in Madison, Wisconsin, USA, as well as ten times in Helsinki, Finland.

The glycogen-glucose and lactate concentrations were analyzed to be 86.0 ± 4.9 mmol/kg and 13.0 ± 1.0 mmol/kg (Madison, Wisconsin, USA) and 85.8 ± 6.9 mmol/kg and 15.7 ± 1.4 mmol/kg (Helsinki, Finland). The correspondent coefficients of variations (CV) where then 8.1, 7.9, 8.0, and 9.2%, respectively.

Methods of assessing meat quality

pH value

Ultimate pH of left *longissimus thoracis et lumborum* (LTL) muscle from Ayrshire bull carcasses (n=57) weighing 279 ± 27 kg was measured (Knick Portamess, Mettler-Toledo 427 electrode) immediately caudal to the 12th rib 48 h after slaughter.

Color

Color was measured from LTL cross-section at three (fresh beef) and two (fried steaks) random spots with a Minolta CR-200 (d65) device (Minolta Camera Co., Japan). The color values L*(lightness), a* (redness) and b* (yellowness) were expressed as means of these three or two measurements.

Drip loss, thawing loss and frying loss

Drip loss was measured according to Honikel (1985). LTL slices weighed 120 ± 18 g (mean ± sd). They were cut 52-58 h *postmortem*, weighed, immediately suspended with a thread, put in a plastic bag, sealed, placed to hang freely at +4 °C for 48 h, and weighed again.

Slices of 15 mm in thickness were cut from semi-frozen meat pieces (thawed at +8 °C for 18 h) for sensory evaluation (V, Fig. 1), weighed, thawed to +18 °C while covered with plastic film, weighed and fried simultaneously on both sides (Palux Rotimat) at 300 °C for 3 min, and weighed again. Percent decrease in weight in thawing and frying were calculated, and weight losses were expressed as means of two or three slices. Sum of the weight losses in thawing and frying was also calculated.

Shear force

Shear values were measured using Instron Universal Testing Machine (Instron Corporation, MA, USA) equipped with a square blade. Meat pieces (V, Fig. 1) were thawed for 24 h at 0°C, but were still frozen when 4 to 6 sample pieces of 2 x 2 x 6 cm^3 (width x height x length) were cut parallel to the fiber orientation. The pieces were then equilibrated to +8 °C, cooked in plastic bags in a water bath (Cryovac ST 5, Grace GmbH, Germany) at 85 °C for 8 min to internal temperature of 70 °C. Pieces were again equilibrated to +8 °C and measured for shear force values at ambient temperature using a 10 or 20 kg load cell at the speed of 20 cm/min. Three shears were performed on each cubic piece. The shear values were expressed as means of these 12 to 18 measurements.

Sensory evaluation and color of steaks

The sensory evaluation of the steaks was performed on fried LTL slices. Each sample (2-3 slices) was evaluated by a trained panel of six members. A continuous segment of a line was used on a scale from 0 to 100 for evaluation of the following variables: resistance to chewing on the first few bites (extremely low/high resistance), overall tenderness (extremely tough/tender), juiciness (extremely dry/juicy), intensity of beef flavor (extremely weak/strong flavor), intensity of fat flavor (extremely weak/strong flavor), intensity of off-flavors (no off-flavor/extremely strong off-flavor), and overall palatability (extremely unpalatable/palatable).

Statistical analyses

Data were analyzed using GLM procedure (I–V), REG procedure (IV), and CORR procedure (IV) of SAS/STAT program (SAS Institute 1990).

SUMMARY OF RESULTS

Glycogen concentration in 8 locations of bovine LTL muscle

The average glycogen concentration of the LTL muscle of Charolais crossbred steers (n=6) in study I varied from 89 to 105 mmol/kg. Cranial-caudal sampling location affected glycogen concentration ($p<0.05$) (I, Table 1). The area of the first lumbar vertebra had more glycogen than the three most cranial ($p<0.005$) and the last caudal ($p<0.05$) locations. When the eight sampling locations were aggregated into three location groups (cranial, mid-section, caudal) (I, Table 2), glycogen concentration of the cranial group (89 ± 3 mmol/kg) was lower ($p<0.005$) than that of the mid-section (97 ± 2 mmol/kg) and caudal (96 ± 2 mmol/kg) groups.

However, the variation of glycolytic potential (GP), a commonly used estimator of resting glycogen concentration, was not significant between the 8 locations nor the aggregated locations (I, Table 1). Nevertheless, the tendency ($p<0.15$) for lower GP at the cranial area compared to mid-section and caudal part remained clear (I, Table 2).

Resting muscle glycogen concentration in American Charolais crossbred bulls and steers, and Finnish Ayrshire bulls

The average resting muscle glycogen concentration in LTL and GM muscles across experiments, dietary treatments, male statuses and seasons were 100 mmol/kg and 112 mmol/kg in American crossbred Charolais cattle (n=38) and 87 mmol/kg and 95 mmol/kg in Finnish Ayrshire bulls (n=60), respectively. The correspondent ranges were 28–157 mmol/kg, 30–187 mmol/kg, 24–135 mmol/kg, and 34–160 mmol/kg, respectively.

The baseline glycogen concentrations across muscles of all-forage fed cattle were 118 mmol/kg in American crossbred Charolais' and 93 mmol/kg in Finnish Ayrshires.

Effects of experimental feeding on resting muscle glycogen concentration

Diet did not consistently affect the glycogen concentration in cattle that had initially high concentrations. Provision of feed high in energy/energy density for cattle previously receiving only forage did not result in increase in glycogen (II, Table 2 (B2, B6); IV, Table 3). On the contrary, the initial glycogen concentration had a very significant negative correlation with the response of glycogen concentration to experimental diet in the Ayrshire bulls ($r = -0.678$, $p<0.0001$) as well as in the Charolais cattle ($r = -0.527$, $p<0.0001$).

Prior to the biopsy sampling B3 (II, Table 1 and 2), the American crossbred Charolais cattle lost glycogen. The magnitude of this loss was 28 mmol/kg on average. However, in cattle that had been receiving the feed high in energy density prior to sampling, this loss was only 8 mmol/kg, whereas in cattle that had been receiving the feed low in energy density, the loss was 46 mmol/kg (II, Fig. 1 and 2).

After adrenaline administration the repletion of the glycogen concentration was faster with the diet high in energy density than with the diet low in energy density. Even after 37 days on diet

(II, Table 3 (B5)) there was significantly more glycogen ($p \leq 0.05$) in the muscles of the corn-fed cattle (119 mmol/kg) compared to the forage-fed cattle (96 mmol/kg) across male status and muscle.

The Finnish Ayrshire bulls on low energy feed during spring lost 17 mmol/kg of glycogen on the 14-day diet and were really the only group that responded to the diet. However, the high and low energy groups of spring and summer did not turn out to be identical in feed consumption. The summer cattle on low energy had such a good appetite compared to the spring cattle that they ultimately got 30% more energy per day from their 100% silage diet.

Effect of muscle, male status and season on resting muscle glycogen concentration

There was less glycogen across diets and seasons in the LTL (88 ± 3 mmol/kg) than GM (97 ± 3 mmol/kg) muscle of Finnish Ayrshire bulls (IV, Table 3). The same appeared to be true for American crossbred Charolais cattle, although it was not rigorously assessed.

Castrated (n=19) and intact males (n=19) did not differ significantly in muscle glycogen concentration nor in the responsiveness to the diets.

Finnish Ayrshire bulls had more resting muscle glycogen in summer (103 ± 3 mmol/kg) (n=30) than in spring (82 ± 3 mmol/kg) (n=30). The difference was still significant after 14 days on experimental diets, and it persisted all the way to slaughter.

Glycogen loss from farm to slaughter

Finnish Ayrshire bulls lost significantly less ($p<0.0001$) glycogen on high-energy diet (11 ± 3 mmol/kg) than on low energy diet (28 ± 3 mmol/kg) between farm and slaughter (included mainly the effect of transportation) (IV, Table 3). Season played a role as well. The cattle transported and slaughtered in spring lost only 15 ± 3 mmol/kg, whereas the summer cattle lost 25 ± 3 mmol/kg.

The cattle transported in spring (–5 °C) lost 7 ± 4.0 (se) mmol/kg and 23 ± 3.9 mmol/kg on high and low energy diets, respectively. The cattle transported and slaughtered in summer (> +25°C), on the other hand, lost 16 ± 3.8 mmol/kg on high-energy diet and 33 ± 4.1 mmol/kg on low-energy diet.

Glycogen loss at transportation had a significant positive correlation with the concentration measured prior to transportation (Gly-diet) ($r=0.649$, $p<0.0001$) as well as with the change in glycogen, while on experimental diet (dGly-diet) ($r=0.505$, $p<0.0001$) (IV, Table 4).

Glycogen concentration at the time of slaughter

The retrospectively calculated concentration of glycogen (= [residual glycogen] including glycogen, G-1-P, G-6-P and free glucose + $[\text{lactic acid}]^{-2}$) at the time of slaughter was in 81 mmol/kg LTL and 100 mmol/kg in GM of Charolais crossbred cattle, across diet and male status, and 65 mmol/kg and 74 mmol/kg, respectively, in Finnish Ayrshire bulls, across diet and season. In the case of the former, the glycogen concentration was independent of diet. However, the latter had more glycogen at the time of slaughter, if they had received the high-energy diet

(73 ± 2 mmol/kg) than if they had received the low energy diet (66 ± 2 mmol/kg). There was a clear trend (II, Table 4) for lower glycogen concentrations in bulls (84 ± 2 mmol/kg) compared to steers (99 ± 3 mmol/kg), although the difference was significant only in LTL.

Ultimate pH and residual glycogen concentration

The relationship between ultimate pH and residual glycogen concentration was curvelinear (III, Fig. 1.) (n=399 across muscles). At normal pH values (≤ 5.75), the glycogen-glucose concentration was independent of pH, while the total range of values was as high as 73 mmol/kg, maximum being 83 and minimum 10 mmol/kg.

All of the Charolais crossbred cattle (n=36) produced meat of normal low ultimate pH, average being 5.47 ± 0.04 (range from 5.40 to 5.62). The residual glycogen concentrations in GM, LTL and SM (*semimembranosus*) were 66 ± 4 mmol/kg, 49 ± 3 mmol/kg and 58 ± 3 mmol/kg, respectively. Steers (64 ± 2 mmol/kg) appeared to have more glycogen left over than bulls (50 ± 2 mmol/kg), but the difference was only significant in LTL.

The average ultimate pH of the meat from Finnish Ayrshire bulls (n=60) was 5.82 ± 0.03 (range from 5.51 to 7.15) and the correspondent residual glycogen concentration was 33 ± 1 mmol/kg. These were independent of muscle and season, but diet affected them very significantly. Bulls on high energy produced meat of lower pH (5.69 ± 0.03) and higher residual glycogen concentration (40 ± 2 mmol/kg) than bulls on low energy, 5.93 ± 0.03 and 27 ± 2 mmol/kg, respectively.

Physical and sensory quality of meat in relation to residual glycogen concentration

The statistically significant effects of residual glycogen on the physical and sensory quality of beef were numerous (V, Tables 2 and 3). Several trends could also be observed in the effects that were not consistently significant.

As residual glycogen concentration increased, the redness (a*) of fresh, oxygenated meat surface decreased, as did the redness of steak. Also drip loss decreased from 4.3% to 3.3% and further to 3%, but the effect failed to reach significance. On the other hand, with increasing residual glycogen concentration, the weight loss in thawing increased as did the calculated sum of weight losses in thawing and frying. Additionally, b* value of steak color increased as the concentration of glycogen increased from below 25 mmol/kg to 25–50 mmol/kg. However, there was no further increase, when the concentration increased to above 50 mmol/kg. Similarly, shear force and the sensory evaluated juiciness of steak decreased as the concentration of glycogen increased from below 25 mmol/kg to 25–50 mmol/kg, but not significantly more with further increase in glycogen.

There was a significant interactive effect of residual glycogen and ageing on the lightness of steak as well as the sensory evaluated off-flavor. When fresh frozen meat was fried as steaks, the steak became lighter as the glycogen concentration increased, and the intensity of detected off-flavors decreased. However, when the steak was fried from the beef aged for 28 days, there was a reduction in lightness, although not consistent, with increasing glycogen concentration. The intensity of off-flavors was strongest in the aged steaks, when there was above 50 mmol/kg glycogen.

DISCUSSION

Resting muscle glycogen concentration in bovines

Justification for repeated sampling of bovine LTL muscle

Skeletal muscle is a heterogeneous collection of cells with different regional populations of fibers of various physiological and biochemical properties (Bruce and Turek, 1985; Suzuki and Tamate, 1988). Tarrant and McVeigh (1979a,b) studied the effect of skeletal muscle needle biopsy on bovine muscle glycogen in cattle and found that the procedure did not affect glycogen metabolism in LTL muscle during the 25-day trial during which each animal was sampled 10 times. The maximum variations from the initial value of 88 mmol/kg were +5 and –9%, and these fluctuations were not significant. Furthermore, they concluded that needle muscle biopsy is useful for the investigation of muscle metabolism in cattle, since it is simple, rapid, and repeatable, and does not cause an unacceptable degree of stress.

Percutaneous needle biopsy is commonly used for obtaining live-animal muscle samples for substrate determinations. As small as 40-80 mg samples (Harriss *et al.*, 1974) have been taken as representative of a whole muscle. To avoid having to speculate about the small sample size as representative for the muscle, Desmecht *et al.* (1995) used larger muscle fragments (2.4–9.5 g) for validation of creatine, L–(+)–lactate and glycogen determinations. They found that when biopsies were carefully taken at resting conditions, methods of expressing lactate and glycogen concentrations (wet matter, dry matter, total creatine) never yielded different trends between sites and muscles. However, they did note that this is probably not the case if muscle works, e.g., during labored breathing in *diaphragma* (D). They also observed that in bovine LTL and D muscles the concentrations of glycogen were always significantly more variable from one site to the other than lactic acid and total creatine.

Karlsson (1971) obtained biopsy samples from different parts of human *Quadriceps femoris* muscle at rest and after sub-maximal and short exhaustive work intensities, and concluded that the distribution of glycogen particles was sufficiently uniform to warrant analysis made on a single biopsy sample taken as representative of the muscle as a whole. Comparably, Desmecht *et al.* (1995) found that the glycogen content was homogenously distributed along bovine LTL muscle, since the results showed no significant systematic difference between sites, and concluded that even if concentration differences were present within muscles, the specimen itself was representative of the muscle investigated.

In this study, considering the fact that GP failed to unambiguously invalidate locational differences in glycogen concentration, one is inclined to believe that these differences may be due to variation in intramuscular fat content. Intramuscular fat has been reported to be higher at the cranial end of the muscle (6th rib) when compared to the 12th/13th rib area (Cook *et al.*, 1964). However, Cross *et al.* (1975) found that marbling was most abundant at the 12th/13th rib interface of LTL muscle when compared to 5th/6th rib interface and loin-round interface. Marbling was more evenly distributed and finer in texture at the 12th/13th rib interface versus the other two locations.

The confounding of muscle metabolite concentrations by adipose and connective tissues as well as blood can be eliminated by carefully removing those before biochemical analyses are performed, a routine of which is used by many muscle scientists (e.g., Essén-Gustavsson *et al.*, 1984). However, this procedure of dissecting freeze-dried muscle samples free from blood, fat

and connective tissue under a microscope in an environment with very low relative humidity is very expensive and time-consuming. Many times biochemical analyses have to be performed on fresh-frozen muscle specimen, and only visible foreign tissue can be rapidly removed at the time of sampling.

In this study, nevertheless, the variation of glycogen concentration was 11.6% on average within the two LTL muscles of an animal, which can be cautiously interpreted to encourage the use of LTL muscle for experiments requiring repeated sampling of a bovine muscle.

Resting muscle glycogen concentration

Marsh (1993) stated in his review paper that "Despite significant advances in knowledge in recent years, we are still far from a complete understanding of the biological mechanisms involved: both those that control the glycogen content of muscle and those that are controlled by it. Feeding level is clearly not the primary determinant of ultimate pH; indeed, it is scarcely a determinant at all".

Taking into account that laboratory intercalibration was performed, and successful, it is probably safe to say that the Charolais crossbred cattle in Wisconsin had higher resting glycogen concentration in LTL and GM muscles than the Ayrshire bulls in Finland. The difference in all-forage-fed cattle (118 mmol/kg vs. 93 mmol/kg) justifies this comparison further, when the potentially superior effect of corn is excluded. This difference of 25 mmol/kg in glycogen concentration would correspond to over 2 h of severe stress (with the average rate of depletion 10-11 mmol/kg/h (McVeigh & Tarrant, 1983; Tarrant & Lacourt, 1984)), or alternatively, to 0.96 pH units *postmortem* (Kivikari, 1996, also see Buffering capacity in the Review of literature)).

However, so many factors were different among the studies in these two countries that it is impossible to know the relative significance of each factor, in retrospect, without being able to perform combined analyses with these factors as reliably identified and quantified cofactors. One can, however, list the potentially relevant differences and speculate with caution. The intentional as well as the unintentional differences that existed between the experiments conducted in the USA and Finland included: climate, breed of cattle, type of rearing, types of feeds offered, experimental setup (handling, habituation), duration of experiment, level of exercise, and opportunity for social interaction with co-specifics as well as with humans.

The difference between Ayrshire and Charolais is not just a breed difference. These breeds also represent the heterogeneous populations of dairy cattle and beef cattle. Dairy cattle seem to have a higher incidence of dark cutting meat (Morrisse *et al.*, 1984; 1985, as cited by Sanz *et al.* (1996)), which could just as well be due to differences in general stress resistance, or temperament, than be a consequence of lower glycogen concentration. Breed and temperament difference in relation to dark-cutting were also studied by Sanz *et al.* (1996) who compared Brown Swiss bulls to a more nervous and hard-to-handle bulls of Pirenaico breed, and found that the glycogen concentration was similar in these two breeds, and despite the apparent difference in temperament, the breed did not influence the incidence of dark-cutting.

The cattle in Wisconsin were housed semi-loosely compared to the tie-stall-housed cattle in Finland, and were therefore able to move around the moderate-sized pen, while the Finnish cattle were completely sedentary throughout the experiment, and throughout most of their lives, for that matter. Cattle that have been reared in close confinement experience less physical

activity than animals reared in large lots or pastures and therefore may have lower initial glycogen levels at slaughter (Hedrick, 1981). Vestergaard *et al.* (2000) found that the muscles of young Friesian bulls produced extensively (Ext) (loose-housed, roughage-based diet) had relatively more slow-contracting fibers, a better vascularization, higher oxidative metabolic potential and a darker meat color compared with muscles of intensively (Int) produced (tie-stall housed, concentrate-fed) bulls. Furthermore, the activity of lactate dehydrogenase (LdH) was lower in the Ext-bulls than in the Int-bulls. The glycogen content of LTL and ST muscles, on the other hand, was lower in the Ext-bulls at 360 kg, but higher at 460 kg compared to the Int-bulls. The determination of different fibre types of the bovine breeds in question would have been interesting and beneficial also in this study, but unfortunately this was not carried out.

The difference in the type of housing brings about additional differences. The Charolais cattle were exposed to seasonal temperatures, while the Ayrshires were housed indoors, and therefore, the surrounding climate had a much greater input in the effects of treatments in Wisconsin compared to Finland. Furthermore, while the Charolais cattle were reared in groups of 4–5 animals in adjacent pens, the tie-stalled Ayrshires were able to socialize with two neighboring animals only. As a consequence to this, the establishment of hierarchy remained totally unfinished among the group of Ayrshires compared to the Charolais', for which it was possible to a much greater extent, ie., among 12–15 animals. Cattle are herd animals, and interference with their natural instinct of dominance establishment, may not be irrelevant to this issue. One should not exclude the possible existence of chronic stress that the Ayrshire bulls may have been suffering from, although the presence and the effects of such stress are not well documented in the literature. Grandin (1992) and Smith *et al.* (1993) identified management practices, such as handling and working facilities that are unique to feedyards, as potentially stressful factors to cattle. Perhaps tie-stall housing could be a factor of that sort as well. Tarrant, in his review article (1989a), briefly mentions that muscle glycogen is also utilized in response to chronic stress.

The Charolais' were definitely able to use more of their muscles on a daily basis. According to Pethick and Rowe (1996), sheep that received exercise training for 5 weeks showed an elevated level of glycogen in muscle at the end of the 10-week experiment. These glycogen loading effects of exercise were not seen after 2 weeks of regular exercise, indicating a strong dependency on the time of exercise training. Their findings were similar in cattle (Pethick *et al.*, 1994). They found that the intake of metabolizable energy was a major determinant of muscle glycogen in barley-supplemented cattle that had received 8 exercise sessions. After five more training sessions, the effect of nutrition was not significant anymore, since the effect of training exercise became more important on the increasing glycogen content than nutrition. The Charolais cattle did receive additional, pre-arranged exercise, when they were slowly accustomed to tolerate human handling and the biopsy sampling procedure in the restraining facility. However, Pethick *et al.* (1995) also reported that there was a small, although significant, reduction in the level of glycogen in the commercial feedlot compared to the individually housed animals. Nevertheless, since the intensity and duration made the pre-arranged exercise of this study rather light, the possible importance of gentle handling and slow customization to the sampling procedure is once again highlighted.

Dietary effects

Resting muscle glycogen concentration

In the present study, the effects of diets of different types and energy contents on the resting levels of glycogen were not by any means dramatic in either country, nor were they consistent throughout the setups. In the USA, diet C (corn; high energy density) tended to result in higher glycogen concentration although this effect was consistent across bulls and steers and both muscles only for B3 (II, Table 2) (see Discussion: Seasonal effects). Male status and interaction between male status and diet were insignificant in all comparisons. GM muscle appeared to be more sensitive than LTL muscle to the diet effect since diet C increased glycogen concentration significantly in six of eight comparisons (B2, B3, B5 and B6, II, Tables 2 and 3) involving GM muscle. In comparison, glycogen concentrations of the LTL muscle responded significantly to diet C in four of eight comparisons. Perhaps this difference in glycogen concentration could at least to some extent be explained by the relative activities of these muscles. Therefore, the relatively higher involvement of GM in general locomotion and exercise enhances the accumulation of the higher resting concentration of glycogen compared to the LTL.

In Finland, on the other hand, the only group responding to the diets of different type and energy content was the spring group of cattle that lost glycogen on the low-energy diet. However, the high and low energy groups of spring and summer did not turn out to be identical in feed consumption. As mentioned earlier, the summer cattle on low energy ate so much better than the spring cattle that they ended up getting 30% more energy per day from their 100% silage diet.

These results suggest that resting muscle glycogen concentration cannot easily be manipulated, since providing a diet high in energy does not inevitably result in increase in glycogen concentration. On the contrary, a very significant negative correlation between the initial glycogen concentration and response of glycogen concentration to diet was found both in the Ayrshire bulls and the Charolais cattle. Initially high glycogen concentrations were not particularly responsive to diet: the more glycogen there was initially, the less was gained during high-energy feeding. Perhaps the synthesis of glycogen is feedback regulated so that the glycogen concentration affects the need to synthesize more, which is typical for the utilization and synthesis of biochemical substrates. Indeed, Tarrant (in a review, 1989a) noted that the level of glycogen regulates the repletion rate in muscle. The lower the absolute concentration of glycogen after stress, the greater the rate of increase after stress.

Furthermore, persistence seems to be a characteristic of skeletal muscle glycogen even when a relatively low energy diet is consumed. This applied to both countries, since the all-forage cattle had relatively high resting concentrations initially, and did not automatically respond to an increase in the energy content of diet. This phenomenon is in accordance with the studies of Howard and Lawrie (1956) and McVeigh and Tarrant (1982), in which glycogen concentration did not decrease dramatically in fasted cattle.

Contrary to these results, Pethick and Rowe (1996) concluded that glycogen is a dynamic metabolite in the skeletal muscle of sheep, and highly responsive to nutrition and exercise training. Pethick *et al.* (1995) also reported that the level of glycogen was dramatically reduced in Australian steers grazing dry pasture in December when compared to those receiving the feedlot ration. Similarly, Daly *et al.* (1999) reported that the glycolytic potential (GP) as an estimate of pre-slaughter glycogen concentration was approximately 20% higher in the LTL

muscle of the corn-fed Angus-cross cattle compared to cattle grazing ryegrass/clover pasture. In their setup, feed intake of the grain-fed steers was restricted with the intention of producing equivalent growth rates in the two treatment groups. However, this attempt was unsuccessful, and during the 6-week trial the grain-fed cattle gained 0.84 kg/d, whereas the pasture-cattle gained 1.90 kg/d. After a thorough discussion, the authors concluded that the difference in GP is very probably due to greater accumulation of muscle glycogen in the steers fed grain concentrates and, thus, suggest that grain-based diets can increase muscle glycogen independent of the higher calorific intake normally associated with grain diets, probably in response to alterations in rumen VFA production. However, the cattle were off-feed for 18 h prior to slaughter, and of this time they were transported for 30 min and laired for 14 h at the abattoir facility. According to the authors, an alternative explanation not to be excluded for the differences in GP is that the two treatment groups had essentially equivalent muscle glycogen concentrations on-farm, but the pre-slaughter handling procedures stimulated greater glycogen depletion in the pasture-fed group. Indeed, the activity of *creatine kinase* and *lactate dehydrogenase* as indicators of pre-slaughter stress were significantly elevated in the pasture-fed steers compared to the grain-fed steers, even though both groups were accustomed to human handling. Based on the experience gained during this study in Finland, especially concerning the losses of muscle glycogen during 5 h transportation followed by immediate slaughter, the alternative explanation of the authors seems more likely (see Discussion: Loss of glycogen from farm to slaughter).

In a study with Merino sheep, Pethick and Rowe (1996) observed a strong linear relationship between the feed intake and glycogen level in the SM and ST muscles. Pethick *et al.* (in a review, 1995) cautiously discourage the usage of LTL or SM muscles for dietary studies, since they may be less responsive to nutrition than the ST muscle. The responsiveness of ST muscle to diet, they say, is closely associated with the inherently low level of glycogen.

Glycogen repletion after induced depletion

The high-energy corn diet was more effective than the low-energy silage diet in repletion of glycogen content in both muscles for both steers and bulls following adrenaline depletion (B5, II, Table 3). There are good indications that feeding practices which supply appropriate gluconeogenic precursors may help to protect animals prior to slaughter or, with rest, to make for more rapid restoration of muscle glycogen in animals that have been stressed (Lister, 1989). However, slow repletion of glycogen concentration occurs even in exhausted animals despite denial of food and water as evidenced by decreasing ultimate pH values (Chrystall *et al.*, 1981).

The rate of repletion was extremely slow, since after 37 days (B5) on diet, the glycogen concentrations in silage-fed cattle were still slightly below the resting concentrations obtained in this study, and there was significantly more glycogen in the corn-fed cattle compared to the silage-fed cattle (II, Table 3). It can be calculated that the rates of repletion in GM muscle were 1.6 and 2.3 mmol/kg/d in steers eating silage and corn, respectively, and 1.1 and 2.4 mmol/kg/d in bulls on those diets, respectively. The comparable rates in LTL muscle were 1.2 and 1.6 mmol/kg/d in steers and 0.8 and 1.4 mmol/kg/d in bulls on silage and corn diets, respectively. Repletion of bovine muscle glycogen with a high-energy barley diet was superior to hay also according to McVeigh and Tarrant (1982). In their study, glycogen concentrations returned to pre-adrenaline-treatment concentrations after 11 d on barley or after 14 d on hay feeding at average rates of 7.6 and 6.1 mmol/kg/d, respectively. However, even fasted heifers gained glycogen at a rate of 1.5 mmol/kg/d. On the other hand, in a study of Crouse *et al.* (1984) muscle glycogen concentration of bulls fasted for 96 h was repleted at the rate of 3 mmol/kg/d

after returning to corn-based diet. The authors anticipated a higher rate, since testosterone has been observed to be important in establishing quantities of muscle glycogen in laboratory animals (Gillespie and Edgerton, 1970, cf. Crouse *et al.*, (1984)). However, while the concentrations in this study as well as in the study of Crouse *et al.* (1984) were around 50 mmol/kg, in the study of McVeigh and Tarrant (1982) they were close to 25 mmol/kg in the hay-fed and fasted heifers and about 40 mmol/kg in the barley-fed heifers. As a consequence to this, McVeigh and Tarrant (1982) were ultimately able to conclude that the lower the absolute concentration of glycogen after stress, the greater the rate of increase after stress.

Tarrant (1989a) suggested, after combining data from McVeigh and Tarrant (1983) and Lacourt and Tarrant (1985), where the results for the rate of recovery of muscle glycogen after adrenaline treatment were only one-third to one-half of the rate after mixed penning, that the rate of glycogen recovery is influenced by the nature of stress. Exposure to adrenaline seems to inhibit muscle glycogen resynthesis by a mechanism that is unclear. Considering the very slow repletion rates of this study, if would have been interesting to measure the change in glycogen concentration at least once or twice during the 37 days. The initial rate of recovery during the first few days may have been significantly higher than the average rate in 37 days.

Seasonal effects

A possible explanation for the loss of glycogen in the Charolais cattle (II, Table 2) was seasonal cooling between the biopsies taken on October 7 (B2) and November 6 (B3), especially since dark-cutting condition has been reported to increase particularly in the fall of the year when sudden changes in environmental temperatures and humidity occur, and possibly coincide with a reduction in feed availability (Munns and Burrell, 1966; Tarrant and Sherington, 1980; Eldridge *et al.*, 1986). The average daily low temperature during 14 d prior to B2 was +5°C, whereas during 14 d prior to B3 it was 0°C. Since ambient temperature and biopsy session were confounded in the study, ambient temperature could not be tested as a covariate. Nevertheless, cold temperatures can cause shivering which is known to deplete glycogen. Climatic stress may increase the energy metabolism of a ruminant by 30–50% (Lister, 1989). It is the rumination process that causes a much greater energy loss, over 6-fold on the average, as heat in ruminants compared with other species. Although under certain circumstances this process may help to maintain body heat and hence have a sparing effect on oxidative metabolism, it represents considerable energy loss, which appears to be difficult to control (Lewis and Hill, 1983). Animals subjected to progressive cold will increase heat production as they pass their so-called lower critical temperature that varies among species but may also vary considerable among individuals of the same species (Andersson and Jónasson, 1993), according to whether it is fasting, fed at maintenance or fully fed, and the metabolic response varies according to the stimulus. It is likely to be the change in temperature, which stimulates heat production and not necessarily the temperature alone (Webster, 1983). Indeed, the phenomenon of cold acclimation involves primarily a shift from shivering to nonshivering thermogenesis during the first 2 to 3 weeks of cold exposure (Andersson and Jónasson, 1993). The cattle on 100% alfalfa diet most probably revealed the effects of cold stress.

Grandin (1992) and Smith *et al.* (1993) reported that the incidence of dark-cutting beef is high during very cold and humid weather as well as in very warm weather or when there are great fluctuations in temperature over short periods of time. In a study of Kreikemeier *et al.* (1998) involving several states of the USA, the proportions of dark-cutters were highest in August

(1.43%), September (1.13%) and October (1.40%) compared with incidences of 0.4 to 0.7% during the other months.

The greater glycogen loss observed in the Ayrshire bulls in summer compared to spring may, therefore, also be due to differences in seasonal temperatures, since the average temperature of the day of transportation and four days prior to transportation (six temperature recordings per each 24 h) were –5.8 ± 0.7 °C and 20.1 ± 2.1 °C in spring and summer, respectively. Extremes in temperature and humidity are effective stressors and can contribute to the dark-cutting condition. Unusually high temperatures and humidity put high demands on the animal's cooler mechanisms. When cattle are transported in hot and humid conditions the level of discomfort and incidence of dark-cutters increases (Hedrick, 1981; Grandin, 1989). This has especially been found to apply to temperatures above 35 °C (Fischer, 1981; Grandin, 1992). Even though the temperatures of this experiment were far less than 35°C, the summer cattle may have suffered from hot weather, since during the 5-h-transportation the temperature was above 25°C, which is unusually high for the Finnish climate.

Loss of glycogen from farm to slaughter

In this study, glycogen loss from farm to slaughter, indicating mainly the effect of transportation, of Finnish Ayrshire bulls had a significant positive correlation with the concentration measured prior to transportation (Gly-diet) as well as with the change in glycogen, while on experimental diet (dGly-diet). Therefore, the more the bulls had glycogen prior to transportation and the more glycogen they had gained during the experimental diet, the more glycogen they lost during transportation. Similar observations have not previously been reported on cattle, but Richter and Galbo (1986) reported that in fast-twitch-red and white rat muscle fibers, increased muscle glycogen levels enhanced the breakdown of glycogen during muscle contraction. Comparable data is not available on American Charolais cattle, since biopsy samples were not obtained at the end of the last feeding trial prior to transportation to slaughter.

Diet markedly affected the glycogen loss during transportation. Considering that it takes approximately 26 mmol of glycogen to lower the pH of 1 kg of bovine LTL muscle by one pH unit (at pH range 5.5 ... 7.0) (Kivikari, 1996) the difference in glycogen loss, which equals to 17 mmol/kg, during transportation (IV, Table 3) in favor of the high energy diet would have corresponded to the average glycogen depletion within 1.5 hours of severe stress, or to 0.65 pH units of pH decline *postmortem*. This result clearly supports the suggestion that good feeding practices that provide appropriate gluconeogenic precursors may protect animals prior to slaughter (Lister, 1989).

Interestingly, while the amount of straight-chain glycogen (A-chains; see Fig. 1 p. 20) that can be rapidly mobilized at any one time regardless of the size of the glycogen molecule is 34.6% (Meléndez-Hevia *et al.*, 1993), the glycogen loss in Finnish bulls on low-energy diet either in spring or summer can be calculated (IV, Table 3, (dGly-diet/Gly-diet)·100%) to correspond 32% and 33%, respectively. There are not a lot of studies concerning glycogen losses during routine transportation to slaughter. In the study of Pethick and Rowe (1996) the decrease in the level of muscle glycogen between the biopsy sample and the sample taken 10 min post-slaughter represents the loss associated with pre-slaughter stressors. Although the level of stress was thought to be minimal, including 1-hour transportation and 0.5–3-hour-lairage, the losses of glycogen corresponded to about 30 mmol/kg or 30–40% of the pre-transport/slaughter level

depending on muscle. The authors suggested that glycogen loss from muscle, even under situations of minimal stress, will be significant and that any further stressors will have heavy impact on glycogen level (Pethick and Rowe, 1996). Similarly, the on-farm glycogen level of steers grazing dry pasture, fasted for 18 h, transported to the abattoir and held for another 18 h off-feed, was compared to glycogen level immediately post-slaughter. The losses in glycogen equaled to about 20 mmol/kg or 30–40% depending on muscle type (Pethick *et al.*, 1995).

Even though covariates modifying the least square means were used in the statistical models of this study, and even though the precision of glycogen analysis may not warrant for this type of speculation, it may not have been a coincidence that the glycogen utilization on low-energy cattle in this study as well as in the studies of Pethick and Rowe (1996) and Pethick *et al.* (1995) was this much but not more. After the rapid hydrolysis of the straight chains of glycogen molecule by *phosphorylase*, the relatively low activity of the debranching enzyme complex probably causes a significant reduction in the rate of glycogen depletion until the branches have been eliminated. This may not be of great relevance to many species, such as human, horse and pig, which utilize glycogen at such a great speed, but it may have value in the energy metabolism of acetate-using muscles of lethargic ruminants.

Knowles *et al.*, (1999) transported steers and heifers of mixed breeds for 14, 21, 26 or 31 hours on trucks with a space allowance of 1.55 m^2 per animal. The animals had constant access to hay and water, and received 1 kg per animal per day of concentrate. Seven days prior to transportation, baseline values of different substrates were determined from blood and muscle specimen (*adductor* (AD), *semimembranosus* (SM), *supraspinatus* (SS), and LTL). A small gradual decline was observed in the glycogen concentration in the AD and SS muscles with increasing journey time, but no measurable changes in the LD and SM muscles. The glycogen levels throughout the experiment (observed from the figure) were approximately 73 mmol/kg (LTL), 90–67 mmol/kg (SS), 98 mmol/kg (SM), and 107 mmol/kg (AD). The ultimate pH values of all muscles were below 5.64, and did not differ from each other. The results suggest, the authors thought, that the journeys were not physically very demanding and did not draw greatly on short-term energy reserves. From the point of view of this study, it would have been interesting, if a group of bulls were included in the study to see, how they might have reacted.

The paradox created by the results of concentration-enhanced utilization of glycogen and the effect of diet, amplifies the protective effect of high-energy diet even further. While the animals on low-energy diet had significantly lower glycogen concentration prior to transportation to slaughter (Gly-diet) and even lost glycogen on diet (dGly-diet) (IV, Table 3, effect of diet), they still ended up loosing more than the high-energy-fed animals during transportation to slaughter. Based on the concentration-enhanced utilization, one might have expected them to loose less than the high-energy cattle. Indeed, the higher amount of glycogen lost during transportation by the low-energy bulls (29.6 ± 3.6%) compared to the high-energy bulls (5.5 ± 3.6%) was equally significant ($p<0.0001$) when expressed in proportions compared to the absolute concentrations. On the other hand, the summer group of bulls, which for some reason had more glycogen than the spring group initially (Gly-rest) as well as prior to transportation (Gly_diet), did also loose more on transportation than the spring cattle. It appears, therefore, that the stimulating effect of low-energy diet on glycogen depletion is so powerful that it overrides the effect of concentration-enhanced utilization.

The effect of summer, however, does not exist anymore, when the analysis of proportional losses (not yet performed for paper IV) is performed with the covariates. Thus, if the glycogen concentrations prior to transportation as well as the change in glycogen while on diet were

considered constant, the bulls lost just as much in spring (17.0 ± 3.7%) than in summer (18.1 ± 3.7%). Therefore, it definitely seems that the increased glycogen loss at transportation in summer compared to spring, revealed the effect of enhanced glycogen utilization in greater glycogen concentrations.

The questions remain: why did high-energy diet protect Finnish Ayrshire bulls from glycogen utilization during transportation? Or alternatively, why did all-roughage diet enhance glycogen utilization despite the lower pre-transportational concentration during transportation to slaughter? And why were the American Charolais crossbred bulls and steers protected against glycogen depletion associated with diet or male status? This issue may well be discussed using the same speculative factors as with discussing the differences in resting muscle glycogen concentration between the Charolais' and Ayrshires.

Well-fed animals are able to withstand the effects of mild stress (Devine *et al.*, 1983), but the effect of cumulative stressors has not received much attention (Devine *et al.*, 1993). Increased ultimate pH (Devine *et al.*, 1993), and in this experiment also the loss of glycogen during transportation, reflect the unsatisfactory imposition of stressors on animals from farm to slaughter. The ease by which different stressors cause metabolic reactions in individual animals and the lack of ability to withstand and adapt to these stressors is directly related to the incidence of dark-cutting (Hedrick, 1981).

According to Franc *et al.* (1986), social friction with the highest agonistic component can be expected in groups of bulls previously tethered individually compared with animals from loose housing conditions. Even though, undoubtedly, this contributes to the differences found between Finland and USA, this is not likely to be a primary cause of glycogen depletion, since the transported bulls in Finland should not be able to move around and "socialize" or fight in the truck nor in the abattoir lairage of single pens. On the contrary, the Charolais cattle were able to fight and move around before transportation, during transportation and after transportation, yet no elevated pH values or even low residual glycogen concentrations were observed. On the other hand, perhaps the Finnish Ayrshire bulls did not have enough room in the truck. After all, carcass bruising and plasma activity of *creatine kinase* have been found to increase with increasing stocking density (Tarrant *et al*., 1988; 1992). However, in a study of Kenny and Tarrant (1987), the motion of the truck was found to be a more stressful component of the transportation than close confinement, as characterized e.g. by heart rate, rectal temperature, and plasma activities of *creatine kinase* and cortisol, and no adverse reaction to loading/unloading was observed. Here, again, the fact that the Charolais' were slowly accustomed to tolerate human handling and the biopsy sampling procedure in the restraining facility, and that they were able to socialize with 12–15 other animals, may have increased their general stress resistance. Furthermore, regular exercise may modify muscles towards increased oxidative activity (Saltin and Rowell, 1980), but this is rather unlikely to have taken place considering the intensity and duration of exercise in the experiments conducted in Wisconsin.

Nevertheless, at this point, one is left with a list of speculative reasons as to what ultimately caused the susceptibility of the Ayrshire bulls on the low-energy diet to glycogen depletion during transportation to slaughter. These explanation candidates include the possible rumen fill-effect associated with great amount of ingested forage creating extra agony, the possible cessation of rumination due to emotional stress, the possible shortage of gluconeogenic precursors in the blood to accommodate the increased energy metabolism, or the possible uncontrolled heat loss associated with the ruminal digestion of forage.

Effect of animal temperament

The subjective temperament rating significantly explained the loss of glycogen in Finnish Ayrshire bulls during transportation, the loss being greater in the aggressive bulls. Furthermore, the temperament correlated significantly with several variables along the experiment (IV, Table 4). The more temperamental the bull the more lactate accumulation during biopsy sampling ($p<0.05$), the less glycogen at the time of slaughter ($p<0.05$), the less residual glycogen ($p<0.05$), the worse the appetite ($p<0.05$), and the less weight gain ($p<0.05$). Voisinet *et al.* (1997b) have also reported of worse daily gains of the most temperamental cattle. In another study of Voisinet *et al.* (1997a) the temperament rating also had a significant effect on the incidence of borderline dark-cutters, which were downgraded by the USDA grader based on visual assessment of lean color.

Even though the correlation between temperament and ultimate pH was not significant in the Ayrshires, the significant correlations with the glycogen concentrations indicate that it was only a matter of time and stress stimuli that pH remained unaffected. From the point of view of production efficiency, ease of handling as well as meat quality, cattle selection according to temperament might be worth considering. Hedrick (1981) suggested that one preventive measure against the dark-cutting problem could be the selection of breeding animals which have no related incidences of dark-cutting and especially animals that have temperament not associated with ready excitability.

In Wisconsin, during EXP-1 (II, unpublished results), the first temperament rating (TEMP-1) (behavior when entering the squeeze chute) of the Charolais cattle at the three biopsy sampling sessions, correlated significantly ($r = 0.160$, $p<0.05$, $n=228$) with the animal's rank order in sampling among its penmates (IN-PEN). The latter, on the other hand, also correlated significantly ($r = 0.229$, $p<0.001$) with lactate accumulation at the biopsy sample (LA). Therefore, the earlier the animal got sampled within its pen the calmer he behaved when he entered the scale, and the less lactate was accumulated in his muscles at the time of sampling. However, in EXP-2, while the significant correlation between TEMP-1 and IN-PEN was still there, the correlation with LA was not, indicating that the animals had gotten increasingly accustomed to the biopsy sampling procedure over time. In both experiments, all four temperament ratings had very significant negative correlations ($r_{min} = –0.359$ in EXP-1 and $r_{min} = –0.270$ in EXP-2, $p<0.0001$) with the animal's appetite for the feed offered during the biopsy sampling procedure. The more restless and aggressive the animal behaved (larger TEMP value), the worse its appetite. Or, alternatively, the worse the animal's appetite the more restlessness and the more aggressive the behavior. At-the-time-of-sampling-appetite also correlated significantly with the type of feed treatment ($r = –0.310$, $p<0.0001$) in both experiments: the all-forage-fed animals having better appetite than the corn-fed animals. If nothing else, the correlations observed in Wisconsin have value in highlighting the importance of randomization in experimental designs.

Ultimate pH and residual glycogen concentration

Relationship between ultimate pH and residual glycogen concentration

The relationship of pH and residual glycogen (III, Fig. 1) seems to follow the same curvelinear shape as that with glycolytic potential, as demonstrated by Fernandez and Gueblez (1992) and Przybylski *et al.* (1993), while the relationship of pH and lactic acid is linear (III, Fig. 2). The

highest residual glycogen concentrations in this data were above 80 mmol/kg, which can be calculated to correspond glycogen concentration of about 125 mmol/kg at the time of slaughter. Resting muscle glycogen concentrations of that level were frequently observed in American crossbred Charolais steers and bulls as well as in Finnish Ayshire bulls. On the other hand, the lowest residual glycogen values at the same pH range from 5.4 to 5.75 were only around 10 mmol/kg, which corresponds to about 55 mmol/kg glycogen at the time of slaughter. The variation of residual glycogen at low pH seems to be enormous. Meat of normal pH (≤5.75) has high residual glycogen on average and differs significantly ($p<0.0001$) from meat of higher pH (III, Table 1). However, the lower the pH the larger the range between observed minimum and maximum residual glycogen concentration.

It is also noteworthy that there were several observations where residual glycogen concentration at a somewhat high pH value was so high that it would have easily been sufficient for an additional pH fall of at least half a unit. For some reason this did not occur. Furthermore, Fig. 1 (III) indicates that a minimum residual glycogen concentration seems to exist. Perhaps the denseness of the core of glycogen molecule is self-limiting in a way, and does not allow further degradation by *phosphorylase*, After all, the limit for the action of *phosphorylase* is a chain of four (1,4-bound) glucose units from every 1,6-bond (at the branching point) (Walker and Whelan, 1960). One can roughly estimate, bearing an error margin of analysis in mind that this "limit value" existed between 5 and 10 mmol/kg (III, Fig. 1). Perhaps proglycogen with the molecular weight of 400,000, being only 1/25 of the molecular weight of full macroglycogen (M=10,000,000) (Lomako et al., 1991) is as small as a glycogen particle can get in a living muscle as well as *postmortem*. It is interesting to speculate that if a maximum amount of full macroglycogen molecules of 12 tiers corresponded to a resting muscle glycogen concentration of 120 mmol/kg in an average bovine animal, the *postmortem* hydrolysis to all-proglycogen level would in the very lowest case result in a residual glycogen concentration of 4.8 mmol/kg. In this case, though, the animal would have lost most of its glycogen already prior to slaughter, since only about 45 mmol/kg is needed for the maximum pH fall.

Ultimate pH values and residual glycogen concentrations in the dietary studies

The low ultimate pH values as well as high residual glycogen concentrations of American Charolais cattle were rather surprising considering that the animals had been transported for several hours and allowed to interact with each other without restriction. Perhaps the training to permit human contact and muscle sampling improved their ability to resist stress associated with transportation, or perhaps they just did not find the transportation particularly stressful. Animals will behave differently depending on their previous experience (Hutson, 1980). Hargreaves and Hutson (1990) reported that several exposures of sheep to gentle handling, including speaking to and touching the animal, reduced the fearfulness of the animals and improved their approachability during subsequent routine handling.

It is obvious that the ultimate pH values and residual glycogen concentrations of Finnish Ayrshire bulls reflected the glycogen differences that were created by the diets already at the farm (IV, Table 3). These differences got amplified at the transportation to slaughter when various stressors were inevitably introduced (IV, Fig. 1). Indeed, the correlations between the amount of silage (sil-kgdm) and compound feed (comp-kgdm) consumed and the glycogen concentration at the time of slaughter (Gly-kill) are stronger, however both significant, than between those and the glycogen response to diet (dGly-diet) (Table 4). The low energy group of cattle in summer was the only one to produce dark-cutting (pH>6.00), but the ultimate pH of 5.85 of the low energy group in spring can also be considered markedly elevated. The

corresponding residual glycogen concentration of the dark-cutting group was 25 mmol/kg (IV, Table 3), which would have easily accommodated an additional pH drop of the remaining 0.5 units, and even then about 12 mmol/kg would have been left over. For some reason this did not occur. Similarly, the average residual glycogen concentrations of the other groups would have been sufficient for the maximum decrease in pH, but again, this did not happen and the glycolysis ceased when pH was 5.69 in the high-energy cattle and 5.85 in the low energy cattle in spring. Pethick and Rowe (1996) found that while decreasing the dietary intake of Merino sheep from above (2.5-fold) maintenance down to maintenance level the ultimate pH values of SM and LD were not much increased, the effect on ST was much greater. The increase of dietary level from maintenance to 2.5 times maintenance increased residual glycogen concentrations linerly from about 14 mmol/kg to 45 mmol/kg in SM and LD muscles, and from virtually non-existent to about 11 mmol/kg in ST. These linear increases were even greater in sheep that had received regular exercise, increasing from about 34 mmol/kg close to 51 mmol/kg. Here again, the increase of residual glycogen was modest in ST, from 6 to 17 mmol/kg. They also reported that in meat ultimately having a pH of 5.6, the range of decrease in glycogen concentration was 0.35 to 1.9 %-units [20 to 110 mmol/kg] during the post-slaughter period of 48 h. However, consumed concentrations above 50 mmol/kg are somewhat in disagreement with the concept of buffering capacity.

In the study of Daly *et al.* (1999) the ultimate pH values produced by pasture- and grain-fed steers were equally low (5.32 and 5.35), but the residual glycogen concentrations were significantly higher in the grain-fed compared to the pasture-fed group of steers. The residual glycogen concentrations were, however, extremely low in their study. Expressed as lactate equivalents, there were 2.4 and 11.9 mmol/kg in the muscles of pasture-fed and grain-fed cattle, respectively. Expressed as glucose, these concentrations equal to 1.2 and 5.95 mmol/kg.

Whereas the ultimate pH values of the Charolais steers and bulls were all low, the corresponding concentrations of residual glycogen were very high. The average concentrations across muscles were 66 mmol/kg in low-energy-fed and 63 mmol/kg in high-energy-fed steers, and 52 mmol/kg and 50 mmol/kg in low- and high-energy-fed bulls, respectively. While the average residual glycogen concentration in Finnish Ayrshires was 33 mmol/kg, ranging from 4 to 83 mmol/kg, the minimum concentrations tended to be at that level in the Charolais cattle (range from 25 to 106 mmol/kg). The maximum residual glycogen concentration of 106 mmol/kg seems enormous, but makes sense, when the maximum resting concentrations of 160-180 mmol/kg are considered. When approximately 45 mmol/kg is consumed in *postmortem* glycolysis of 1.73 pH units (from 7.2 to 5.47), a glycogen buffer of 9 to 29 mmol/kg has yet been in excess to be used between farm and slaughter. Contrary to the Finnish results, the highest maximum residual glycogen concentration was in the group of steers fed low energy (100% alfalfa haylage) prior to the transportation, while the lowest maximum was in the group of bulls fed the high-energy ration of corn. However, the residual glycogen concentrations of the Charolais cattle were completely independent of diet and varied more according to the male status.

The reasons for the marked difference in the ranges of ultimate pH values as well as in residual glycogen concentrations between the two countries of this study are worth of some consideration. Although all the same explanation candidates apply to this discussion as to the differences in resting muscle glycogen concentration in general, some deserve additional attention. It seems not likely that the primary causes for the differences lie in the energy content of the diets, since in that regard, the low and high-energy diets of the countries were quite compatible. Nor is it likely that the type of transportation makes all the difference. According to

Eldridge and Winfield (1988), cattle transported at low space allowances had 3.9-6.7% lower heart rates and 127-260% lower movement scores than the cattle transported at higher space allowances. In this study the space allowance of the somewhat tightly-packed Ayrshires was comparable to low, while the loose-compartment transportation of the Charolais' was similar to the high space allowances.

The most potential contributors to the differences are perhaps again the fact that the American Charolais cattle were accustomed to human handling, had participated in several studies over the course of one year, and as a consequence to their gained experience, just did not find transportation to the slaughter particularly stressful. In addition to this, the semi-loose housing of the Charolais' cattle versus the tie-stall housing of the Ayrshire bulls, and the opportunity of the Charolais' to exercise regularly compared to the Ayrshires that had to remain completely stationary may have been relevant.

Independent quality effects of residual glycogen at ultimate pH below 5.75

The mean pH values of all residual glycogen categories (V, Table 1) are so low that they could all be endpoints to a *postmortem* acidification of about 1.5 pH-units. Using the mean buffering capacity of 51 mmol LA /(pH kg) (at pH range from 5.5 to 7.0) of beef LTL muscle (Kivikari, 1996) these pH values would have required about 38 mmol of glycogen at the time of slaughter to be formed. Thus, the correspondent glycogen categories at the time of slaughter would have been ≤ 63 mmol/kg, 63.1–87.9 mmol/kg, and ≥ 88 mmol/kg.

Even though residual glycogen by itself or in interaction with ageing had various statistically significant effects on the physical and sensory quality of beef (V, Tables 2 and 3), ie., juiciness, shear force, thawing loss, frying loss, fresh meat color as well as fried meat color, the independent effects of residual glycogen on beef quality are hardly prominent. Nevertheless, the water holding capacity (WHC) of beef, as characterized with drip loss of fresh meat, juiciness of steak as well as weight losses in thawing and frying, was somewhat affected by residual glycogen concentration (V, Fig. 3). This is not a surprise considering the data from the porcine research (Monin and Sellier, 1985; Lundström *et al.*, 1998). However, in beef having normal ultimate pH (5.50 ... 5.75), the effects were slightly different from the effects on pork.

Whereas the drip loss (or another measure of WHC of fresh meat) of pork having high residual glycogen concentration has been found to be high (low WHC) (Monin and Sellier, 1985; Lundström *et al.*, 1998), the drip losses of beef having high or intermediate residual glycogen concentration (V, Table 2) clearly tended to be lower than that of low residual glycogen ($p<0.061$). At least in fresh beef with this range of ultimate pH values glycogen molecule seems to be able hold on to its bound water. It is noteworthy that in the study of Lundström *et al.* (1998) the pork groups that differed in residual glycogen concentration (21 and 58 mmmol/kg) and drip loss (3.8% and 5.1%) had also different ultimate pH values of 5.51 and 5.42. Perhaps the effect of ultimate pHs being closer to the isoelectric point of meat contributes to this discrepancy, since in this study not only were the pHs slightly higher, but also not all three residual glycogen groups differed in average pH (V, Table 1).

The thawing loss of beef was significantly affected by the residual glycogen concentration. At thawing, or perhaps already at freezing, the water bound in glycogen molecules seemed to become loose. There was no obvious explanation as to why this would happen. Although the effect of residual glycogen on frying loss was insignificant, the sum of thawing and frying losses

was significantly affected. This is well in accordance with the results of Fernandez *et al.* (1991) who measured lower technological yield of Paris Ham (cured and cooked) on samples with high residual glycogen.

The lower shear force values of the high and intermediate glycogen categories compared to the low glycogen category agree with the results of Lundström *et al.* (1996) who reported of lower shear force values on pork from RN^- carriers which typically have high residual glycogen content compared to non-carriers with lower glycogen content.

Residual glycogen categories did not differ in sensory evaluation of beef and fat flavor. Nevertheless, the increasing b* value of steak color could be considered as an indication of the enhanced Maillard reactions during frying (V, Table 3). The high off-flavor rating on the aged samples with the most residual glycogen is surprising. One possible explanation is that high acidity and abundance of lactic acid bacteria may have made a contribution. Unfortunately pH values were not measured again after thawing of the aged and frozen samples, and no microbiological evaluations were done, so there is no way to be sure.

Concept of medium-stress beef

From the pre-slaughter stress point of view the large variation of residual glycogen, even at the lowest pH values, presents an interesting concept (III, Fig. 3). The stress experienced by the animals having a combination of low pH and low residual glycogen (area II) has obviously not been severe enough to ultimately produce elevated pH values or dark-cutting. Yet they have clearly experienced stress of some level, since the glycogen concentrations at the time of slaughter have been below the typical resting glycogen concentration of bovine muscles, ie., 80 to 100 mmol/kg (McVeigh and Tarrant, 1982). The phenomenon could be described as the medium-stress beef (II) as opposed to the low-stress (area I) and high-stress beef (area III) (III, Fig. 3). This seems particularly justified since the lowest residual glycogen concentrations of the area II are exactly comparable to the residual glycogen concentrations of the most severe dark-cutters in the area III. Newton and Gill (1978) presented a table concerning the concentration ranges of glucose in DFD-suspected beef striploins (LTL). The concentrations of glucose seem to follow the same pattern, since the lowest concentrations of glucose in pH groups of 5.60–5.69, 5.7–5.79 and 5.8–5.89, ie., 33, 25 and 10 μg/g, respectively, were equal to the highest concentrations of glucose in four pH groups of the range 6.00–6.39.

What sort of problem does this medium-stress beef possess then? It is well known that meat of high pH (area III) has poor shelf life. Problems associated with microbiological stability may also arise in the case of medium-stress ground beef, since grinding increases the relative proportion of surface many-fold, causes further contamination, and spreads the surface flora allover the meat. In large meat pieces most of the residual glycogen may not be of much use for the microbial flora, since the hydrolysis of glycogen and the diffusion of glucose from the interior of the piece is too slow (Gill, 1976; Kress-Rogers *et al.*, 1993). In ground meat, however, high residual glycogen could be a great advantage, and a very low residual glycogen, on the other hand, problematic. Shelef (1977) mixed ground beef with anhydrous D-glucose at levels of 0.5, 1.0, 1.5, 2.0, 5.0 and 10.0%. Samples with less than 2% glucose spoiled in his experiment in a manner similar to control samples (Table 4), but glucose additions of 2% and above declined the initial pH during early storage and thus, markedly prolonged the shelf life of ground beef. One may speculate with the reasons to why glucose did not have this effect at lower

levels, ie., 0.5, 1.0 and 1.5%. The average pH of the meat was 5.8, which cannot be considered low. The meat had been obtained from retail stores and some of it was also store-ground. It is thus likely that the samples carried a somewhat heavy bacterial load already before the experiment was initiated by adding the glucose. At the present study, the maximum residual glycogen concentration of 83 mmol/kg and the minimum of 10 mmol/kg at low pH values (<5.6) correspond to 1.5 and 0.2%. How these naturally occurring concentrations may affect the shelf life of ground beef, calls for further experimentation.

Table 4. Effect of glucose additions to ground beef on pH[a] (Shelef, 1977)

	Glucose concentration (% by wt)			
	0	2	5	10
Minimum pH value of meat	5.6	5.2[b]	5.1[b]	4.8[b]
No. days required to reach minimum pH	0	9	11	21
No. days required to reach pH 6.0 (onset of spoilage)	5	14	20	25

[a] Mean values for 8 different samples
[b] Significantly different from the control samples ($p<0.001$)

In addition to the potential effects associated with shelf life, the medium-stress beef from the edge of normal ultimate pH, ie., around pH 5.7–5.8, is likely to have representation in the population of tough beef known to be related to moderately elevated, intermediate pH values (5.8–6.2) (e.g., Devine *et al.*, 1993; Purchas and Yan, 1997; Purchas *et al.*, 1999). Devine *et al.* (1993) subjected lambs to various combinations of pre-slaughter stress regimen, such as shearing, restricted feed, and swim washing. The induced stress levels were characterized as low, low/medium, medium, high, and very high, and they resulted in corresponding ultimate pH values of 5.50, 5.84, 5.88, 6.29, and 6.38, respectively. The shear forces of the low/medium (13.2 kgF) and medium (12.4 kgF) stressed groups were significantly higher compared to the other groups of low (9.35 kgF), high (9.17 kgF) and very high (10.5 kgF) stress levels (Devine *et al.*, 1993).

The circumstances resulting in the development of medium-stress beef may, therefore, potentially have several important and far-reaching effects on the ultimate quality of beef in the forms of shelf-life, tenderness and overall palatability.

CONCLUSIONS

- ✓ It is justified to use the LTL muscle of a bovine animal in repeated biopsy sampling on the longitudinal axis provided that the most cranial part of the muscle is avoided and that care is taken to obtain the samples while the muscle is at resting state.

- ✓ Resting American Charolais cross-bred cattle that are housed semi-loosely with a few co-specifics, are exposed to ambient temperatures, are accustomed to human handling, and are exercised regularly (but not intensively), have more glycogen in their LTL and GM muscles than resting, sedentary Finnish Ayrshire bulls that are individually tied in stalls indoors, unaccustomed to handling, and unable to interact with many co-specifics.

- ✓ Bovine muscle glycogen concentration cannot be easily manipulated with diets. Initially high concentrations do not readily respond to changes in the energy content of the diet. The more there is glycogen initially, the less it increases with high-energy diet.
- ✓ Cattle are able to gain and maintain high glycogen concentrations even on an all-forage diet, but the repletion of low muscle glycogen concentrations is faster with feeds high in energy than with low-energy forage.
- ✓ Glycogen utilization in bovine muscle is enhanced by high glycogen concentrations.
- ✓ Consumption of diet high in energy protects bovine animals from potentially glycogen-depleting stressors, such as onset of cold or hot weather, and transportation to slaughter.
- ✓ Finishing of dairy bulls with a high-energy concentrate diet for at least two weeks prior to transportation to slaughter is one of the appropriate measures to take against bovine dark-cutting, since its protective effects were directed against glycogen depletion and elevation of ultimate pH.
- ✓ The most aggressive bulls have worse weight gains, utilize more glycogen at transportation to slaughter, and are more prone to elevation of ultimate pH than normal bulls.
- ✓ Relationship between residual glycogen concentration and ultimate pH of beef is curvelinear, and independent of pH at normal pH values (≤5.75), where the range of glycogen concentration is enormous.
- ✓ Independent effects of residual glycogen concentration on the physical and sensory quality of beef are numerous, but hardly prominent.

SUGGESTIONS FOR FUTURE EXPERIMENTS

- ✓ How the great, naturally occurring variation in residual glycogen affects the shelf life of beef, especially after it has been ground.
- ✓ How does refrigeration temperatures and gradually declining pH affect the *postmortem* activity of the glycogen debranching enzyme complex.
- ✓ What is the relationship between the concentration/debranching state of glycogen at slaughter and the rate and extent of *postmortem* glycolysis.

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