# The Welfare of Cull Holstein Cows in an Abattoir: *Testing the Effects of Reduced Noise in the Chute and a Workshop on Humane Handling*

**by**
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## Dedication

To my loving mother, Enid Robertson
who taught me that the love of animals is the true meaning of wealth

&

To the memory of Greedy, Clara, and all the other wonderful cows
with whom I spent the best sixteen years of my life

“ The greatness of a nation and its moral progress can be judged
by the way its animals are treated. ”

Mahatma Gandhi

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| | |
|---|---|
| BCS | Body Condition Score |
| pH 45 | pH of *longissimus dorsi* muscle of carcass at 45 minutes *post mortem* |
| pHu | pH ultimate of *longissimus dorsi* muscle of carcass |
| CPK | Creatine phospho-kinase |
| PCV | Packed cell volume |
| DFD | Dark firm dry |
| PSE | Pale soft exudative |
| LD | Longissimus dorsi |
| DW | Dressed weight of carcass |
| GLM | General linear model |
| SFC | Single file chute |

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## Abstract

During pre-slaughter handling at a commercial abattoir, cattle are exposed to many potential stressors, among them loud noises and electric prodding. Air-relief valves on pneumatic doors that separate small groups of cows in a single file chute produce a sudden, loud hissing noise as they are raised and lowered between cows. Electric prodding of cows is used to manoeuver them from lairage pens into the single file chute leading to the stunning-box**,** where they are stunned by a captive-bolt pistol prior to exsanguination and death. Cull dairy cows are a high-risk category of livestock for welfare problems, as they generally arrive at an abattoir in poor body condition. A study was carried out to describe pre-stunning behaviour and characteristics of cull Holstein cows from the Atlantic region. This study investigated 1) the characteristic features of a population of cull dairy cows at the time of slaughter, 2) the effects of reducing the noise of air-exhaust valves on vertical doors in the single file chute, and 3) the effects of a workshop with abattoir staff aimed at reducing electric prodding of cows. Seven hundred and fifty two (752) cull Holstein cows were observed as they were handled in the stunning-box area just prior to slaughter, at a large abattoir in the region. Ten responses (5 behavioural and 5 physiological) were used as indicators of fear and stress.

Using zero-one sampling across all treatments average frequencies of 58.4% balking, 50% struggling, 29.4% attempting to turn around, 36.6% trembling, and 7.4% vocalizing were observed (n=752). Exsanguinated blood showed an average PCV% of 35.8±0.24 (n=625), 22.2±1.0 ng/ml plasma cortisol (n=209), and 684.7±55.4 U/L creatine phospho-kinase (n=209). The *longissimus dorsi* muscle of the carcasses showed an average pH of 6.76±0.01 at 45 min *post mortem* (n=534), and 6.14±0.01 at 72 hours *post mortem*. Forty-one point eight percent (41.8%) of the cows were emaciated (BCS=1, n=744), 82.4% were lactating, 15.6% were pregnant, and 65% of the carcasses were classified as dark-cutters (pH ultimate > 6.0).

The installation of mufflers to reduce noise of air-relief valves on pneumatic doors in the chute did not appear to make a significant difference in the cows' behaviour. A workshop on humane handling of cattle led to a 30% reduction in the use of the electric prod from 96% to 67% (P<0.0001, n=752), and a 20% reduction in the time the cow spent in the single file chute (P<0.0001, n=579) in the six weeks following the workshop. It also led to a 14.2% reduction in balking (P<0.05), a 22.5% reduction in attempts to turn around in the single file chute (P=0.08), a 3.9% decrease in PCV% (P<0.01) due possibly to reduced splenic contractions, no change in cortisol, a 1.5% increase in pH at 45 min *post mortem* (P<0.0001) due possibly to reduced acute fear, and no change in pH ultimate. It is suggested that initial training of staff at an abattoir led to small yet positive improvements in the welfare of cull Holstein cows during the immediate pre-slaughter period.

# Chapter 1. Introduction

## 1.1 The Culling of Dairy Cows and Welfare Risk

Based on the Atlantic Dairy Livestock Improvement Corporation's (ADLIC) 1997 report, approximately 16,400 Holstein cows (*Bos primigenius taurus*) are culled each year from Atlantic dairy herds, and the majority (81.5%) of these cows are destined for the abattoir. No formal ethological survey has yet been conducted, to my knowledge, that has examined the entire process by which these cows are removed from the farm and are transported and sold eventually to an abattoir where they are slaughtered for meat. No epidemiological survey conducted with this category of livestock in abattoirs was found.

There is little financial incentive to handle cull dairy cows in such a way as to minimize the stress they experience during the pre-slaughter period because their carcasses are used for low-value meat products. A cull dairy cow typically experiences a huge range of physical and psychological stressors during the entire culling process, beginning with her removal from familiar herdmates and home-farm environment and ending with a series of aversive stimuli such as electric prodding and sudden loud noises among unfamiliar cows. Consequently, it can be expected that a dairy cow's normal behaviour is severely altered and her welfare compromised.

Both dairy industry and public initiatives have indicated an ethical belief that animals must not be treated in any way that causes unnecessary suffering, pain, injury, or death. For instance, the Recommended Code of Practice for the Care and Handling of Dairy Cattle (Agriculture Canada, 1990), states that " all personnel working with cattle should understand and accept their responsibility to prevent avoidable suffering of animals", and that "sudden changes in environment should be minimized during transportation, and dairy cows should not be subjected to excessive, unfamiliar noise". The Federal Meat Inspection Act, Part 3, Section 62.1 (1990) states that "no food animal shall be handled in a manner that subjects the animal to avoidable distress or avoidable pain", and the Act provides for criminal prosecution where its regulations are violated.

Dairy cows are a distinct population group since they typically experience closer associations with humans throughout their lifetimes than do other commercial cattle types, such as beef cows and feed-lot cattle. Dairy cows learn to cope with a large range and variety of stimuli in their normal environment, and become habituated to many complex routines involving noisy equipment and human contact. How well equipped these cows are, as a result of this habituation, to adapt to a series of environmental and social changes constituted by the culling process whereby they end up killed in a slaughterhouse, has not been a subject of research to date. In addition, a cull dairy cow may or may not be lactating at the time she is shipped for slaughter, which presents a distinctive characteristic of an animal in a slaughterhouse setting which hitherto has not been studied from an applied ethology perspective. For these and other reasons, such dairy cows who are in a pre-slaughter stress situation constitute a unique animal population in terms of welfare risk.

Once inside an abattoir, cull dairy cows face the same physical conditions as all cattle there. However, this does not mean that they are handled in the same way as younger, healthy fed beef cattle, nor that their experience is the same.

Pre-slaughter handling stress in beef cattle has been studied by several workers in the last twenty years. However, these studies do not always provide details as to cattle type, use very different cattle types in surveys or to test treatments, and have used very few animals in a sample. In some, potential stressors are identified but not examined experimentally. Cull dairy cows as a specific cattle type have not been studied in the context of pre-slaughter handling behaviour and/or carcass characteristics.

For these reasons, it was felt that a behavioural study of a fairly large sample of cull Holstein cows, using non-invasive methods of both behavioural observation and physiological measurement, investigating quantifiable effects of reducing loud noise and electric prodding of cows, would shed some light on the degree to which this type of cattle suffer during the immediate pre-slaughter handling period at an abattoir.

## 1.2 Objectives

The goal of the study was to test the hypothesis that a reduction in the sudden, loud noise produced by air-exhaust valves on pneumatic doors in the single file chute at an abattoir, and training of employees in the humane handling of cattle, would result in observable differences (relative to the status quo) in the fear behaviour of cull Holstein cows at the point of slaughter, thus improving their welfare at this critical time. In the process, it was hoped that some insights could be gained into characteristics of this specific category of cattle, and their typical behaviour in an abattoir just before slaughter.

Objective # 1: To determine the effect of installing mufflers on noisy pneumatic doors in the single file chute on pre-slaughter behaviour and post-slaughter carcass characteristics of cull Holstein cows.

Objective # 2: To evaluate the effect of a humane handling workshop with abattoir employees who worked directly with the animals on the pre-slaughter behaviour and post-slaughter carcass characteristics of cull Holstein cows.

Objective # 3: To describe some characteristic features of a population of cull Holstein cows from the Atlantic region, at the time of slaughter at an abattoir.

# Chapter 2. Background and Literature Review

## 2.1 Composition of Cull Cow Population

‘Culling’ refers to the removal of a cow from a herd at any stage of lactation, and includes death, sales for dairy purposes, and slaughter. Reasons for culling appear to have stayed approximately the same over time in this part of the world, while culling rates have increased dramatically since the 1980s. Milian-Suazo et al. (1988) found a culling rate in New York State herds between the years 1981-1985, similar to earlier studies, to be 18.7% per year, and that the primary reasons for culling were failure to conceive, udder problems, and low milk production, but also included feet and leg problems, old age, accidents, and other “miscellaneous” reasons. The peripartum period was shown to be the most critical regarding survival of the dairy cow, and therefore correspondingly the highest risk period for culling. Culling peaked immediately after calving for any of the main three reasons mentioned above, in mid to late lactation for low milk production, and late in lactation for reproductive problems. Culling increased with parity, and some culling was “forced” because of disease such as milk fever, downer cow syndrome, left displaced abomasum, and teat problems, and many cows were culled immediately or within 30 days of the diagnosis. Cows diagnosed with diseases such as retained placenta, metritis, mastitis, and cystic ovaries, were kept longer before culling. Mature cows comprised 82% of culls. A more recent study carried out in 1996 in New England, U.S.A., (Bascom and Young, 1998), found that failure to conceive (20 %), mastitis (15%), and low milk production(14%) were the three main reasons in order of importance, that dairy farmers culled cows from their herds. These workers also reported that many cows were culled for multiple reasons, and that there is variation among farmers in their culling decision-making. Based on Dairy Herd Improvement Association (DHIA) records for the Northeastern U.S., culling rates averaged 34% in 1995, up 82% from the 1980s study. In a recent Quebec Dairy Herds Health Improvement (ASTLQ) Project study, Bouchard et al. (1997) found the average culling rate for 1996 to be 30.1%, and the three main identified reasons for culling were, once again, reproductive failure (30.9%; sub-divided into metritis 15%, ovarian cysts 9.6%, and abortion/embryonic death 5.2%), mastitis (26.4%), and low milk production (18.9%).

In general, dairy farmers aim for a calving interval of 12 months, and so aim to breed their cows back within 90 days of calving. A non-pregnancy diagnosis half-way through the lactation (150 days post partum) makes the cow a cull candidate. Bouchard et al. (1997) found that the average interval from calving to culling, for cows with reproductive problems, was 174 days. The 1997 Atlantic Dairy Livestock Improvement Corporation's (ADLIC) report listed reproductive problems (24.4%), sold for dairy purposes (17.8%), low milk production (16.5%), and mastitis (12.4%), as the primary reasons for culling. Many other reasons, such as feet and leg problems, udder problems, 'other', injury, sickness, old age, were stated, but occurred less often.

Although consistent in their reporting of reasons for culling, none of these reports investigated how many of the cull cows actually were sold for slaughter following removal from the farm. No published literature on the composition and epidemiology of the cull dairy cow population arriving at the abattoir was found. An examination of the ADLIC report for 1997, however, suggests that approximately 81.5% of cows disposed of live were sold for slaughter.

On December 31, 2000, formal tracking of both dairy and beef animals will begin in Canada with the National Livestock Identification (NLID) program (Anon, 1999). Designed as a means of quick and effective response in the event of a human health emergency which originates from eating beef, this program may also help in improving accountability in the handling and care of cull cows throughout the marketing process up to slaughter.

## 2.2 Condition and Quality of Cull Dairy Cows at Slaughter

One indication of a difference between the physical condition of fed (beef steers and heifers) versus non-fed (cull cows and bulls of dairy and beef breeds) cattle arriving at slaughter plants in Canada, is that cull cows and bulls have four times the injection site damage in sub-primal cuts than fed beef cattle. In a survey of injection site lesions in cull cows and bulls, funded by the Canadian Cattlemen's Association in 1997, Van Donkersgoed et al. (1998) estimated that 649,702 cull cows and bulls were processed in Canada in 1996, and that 35% of them had injection-site lesions on their outside rounds. This compares with 8% occurrence in fed cattle. The proportion of beef and dairy cows was not determined, but an estimate from purveyors was that 70% of the cows were dairy cows. In a similar study, the 1994 U.S. National Non-Fed Beef Quality Audit (Colorado State University) showed that bruising in cull cows was excessively high, with 31% of all cows slaughtered having major bruises. This compares with 5% of beef steers and heifers, reported in the 1992 National Fed Beef Quality Audit. Thin animals were found to bruise more easily than fat animals. Some of these bruises were shown to be sustained due to handling in the chute, such as severe back bruises caused by closing vertical doors on the animals' backs. This report cited that 4.7% of dairy cows were found to be lame, and very skinny "bone-rack" (emaciated) cows were identified as a problem which "should be addressed by the dairy industry" by marketing cull cows sooner rather than later.

**2.3 Pre-Slaughter Stress**

Kilgour (1978) identified the need for research attention to the stress and discomfort that animals experience during the lairage period and the movement of animals to the point of stunning prior to death. Grandin (1996) suggested that the five main causes of animal welfare problems in abattoirs are poor condition of animals, lack of employee training in principles of animal behaviour and methods of humane handling, physical distractions that impede movement of animals as they are handled (such as high-pitched noise and air-drafts in chutes blowing in animals' faces), stressful equipment and handling methods, and poor maintenance of equipment such as captive bolt pistols for stunning cattle, and slippery flooring.

Tume and Shaw (1992a) compared beta-endorphin and cortisol concentrations in exsanguinated blood of two non-equivalent groups of cattle. They used 36 adult cattle of mixed breeds who were rested on site for a period of 1-4 weeks prior to being slaughtered at a quiet research abattoir, and compared them with 36 bullocks who had been transported a distance of 200 km and then rested for 18 hours prior to slaughter at a commercial abattoir. They found higher cortisol values in the animals slaughtered in the commercial abattoir, but no dark-cutting carcasses in the group. Mitchell et al. (1988) used 17 "unstressed" dairy cows of two different breeds (Friesland and Nguni) as a control group for an experiment with 80 cross-bred beef steers and heifers from a feedlot, in which only physiological responses were used to assess stress following handling, transport and slaughter. This study found that packed cell volume (PCV) % was significantly higher following handling and slaughter as compared to control values. It concluded that the increase was possibly due to splenic contractions in stress, even though the authors did not find any increase in norepinephrine concentration.

Dunn (1990) compared stress reactions of cattle who underwent ritual slaughter using either the Weinberg pen (in which the animal is inverted 180° before having the throat cut) or the American Society for the Prevention of Cruelty (ASPCA) pen (in which the animal is standing). This worker found greater indications of stress (increased values of cortisol and PCV%) experienced by the cattle killed in the inverted pen, as compared to the ASPCA pen or conventional slaughter. However, he tested these different methods of restraint in two different abattoirs in two different countries, thereby introducing an increased risk of the presence of confounding background factors. The experiment used mature beef cattle of "random breed and sex" for the experiment. Data for the control group (slaughtered conventionally by stunning with a captive bolt pistol followed by exsanguination) and the group slaughtered in the Weinberg pen, were collected in a meat plant in Scotland, while data for the cattle slaughtered in the ASPCA pen were collected in Northern Ireland. Another study also concerned with the stress of ritual slaughter compared exsanguination of the animal while standing upright to that while inverted. Petty et al. (1994) found that there was no difference in stress (as measured only by blood variables including concentrations of catecholamines, cortisol, glucose, lactate and total lipids). They did not specify (other than that they were *Bos indicus* type) sex, age or breed of animals. Also, they used a small sample of cattle in their experimental groups (n=7-30). In addition to illustrating the lack of attention to potential differences in stress responses according to cattle type and other characteristics such as rearing conditions, these last two examples also serve to point out that the criteria used to determine "stress" at slaughter are different, and therefore very different conclusions have been drawn from studies of the same question.

Cull beef cows were among 220 cattle studied by Jarvis et al. (1996) in relation to handling, bruising and dehydration at the time of slaughter, but there is no indication in this study that any comparisons were made among cattle types. Cull beef cows have been studied as a distinct type in only a few studies. For instance, Wythes et al. (1988b) found that beef cows who were transported directly to an abattoir or were rested twice during a rail journey to the abattoir, tended to show more bruising than cows who were rested only once during the rail journey.

Aside from studies of "downer" (non-ambulatory) cows (e.g., Grandin 1997a), which is a distinct category of cattle within the larger category of cull cows, less is known about factors present during various conditions during the immediate pre-slaughter period which influence various types of stress in cull dairy cows as a type of cattle. Grandin (1997a) is one of the only workers to report data for cull dairy cows (as a type) at the time of slaughter. In a survey of stunning and handling in American, federally inspected beef, veal, pork, and sheep slaughter plants, Grandin found that cows (50:50 beef and dairy types) were distracted by air hissing from control valves, reflections of light, and seeing movement under the stunning box door, of the cow ahead. In the same survey, she found that 35% of Holstein cows (n=20) who were restrained in an ASPCA pen for ritual slaughter at one abattoir vocalized versus 5% in beef cattle restrained in the same pen (n=u/k), and 6% of all types in the survey (n=1320+). In this case, the restrainer had been designed for cattle who are shorter in length than Holstein cows, and consequently the pen was too small for the Holsteins and they were cramped in, causing a higher frequency of vocalization. This report is one example of the importance of appropriate equipment for different types of cattle handled at abattoirs.

Previous work has consistently identified loud noise and electric prodding as causes of stress and fear in cattle handled in the pre-slaughter period, yet the effects of these potential stressors on the welfare of cattle have not yet been quantified in the context of immediate pre-slaughter handling of cattle. Grandin (1996) indicated that 27% of Canadian slaughterhouses had high-pitched motor noise or hissing air that caused animals to balk, however this stressor was not examined experimentally in the study. Orihuela and Solano (1994) suggested that one factor that caused increased agitation in the chute was the increase in noise in the stunning area as the slaughter process progressed, yet its effects were not quantified. However, it appears from other studies that noise is a significant stressor. For instance, in a study of behavioural and physiological responses of pigs to sound (Talling et al., 1996), in which the effects of noise were quantified, it was found that exposure to sound in general changed the behaviour of pigs from resting to aroused and attentive, the expression of the response depended on the properties of the sound stimuli (e.g., higher heart-rates and ambulation scores were recorded for higher sound power levels (97dB compared to 85dB)) and it concluded that abrupt changes in the sound environment should be eliminated for smooth handling of pigs. A study that found a positive correlation between subjective and objective assessments of stress at slaughter in predicting meat quality in pigs (Warriss et al., 1994) also found that the most stressful systems for pigs also produced higher levels of sound, and that there was a positive relationship between sound level and indices of poorer meat quality.

Grandin (1983, 1994a, 1997a) observed that excessive stress was consistently caused by improper use of electric prods on livestock. Cockram and Corley (1991) identified the routine use of driving instruments and delays causing stoppages in the chute as stressors in a slaughterhouse, and recommended using means of handling cattle other than with an electric prod. Cortisol concentration was positively correlated with the time spent standing still and time spent in the pre-stun pen; no correlation was found between physical activity in the chute and creatine phospho-kinase (CPK) levels; and the frequencies of struggling and defecation were greatest while confined in the chute and pre-stun pen. This study sought to quantify pre-slaughter behaviour and handling of cattle in a commercial abattoir, and made detailed observations of 60 cattle (one from each small group of 6) of mixed breeding as they were handled from lairage pens to the stunning pen over a five-week period. Although the focal animal observations, including 9 behavioural states (such as standing still and lying down) and 7 behavioural events (such as vocalization and struggling) were very detailed, these workers observed a mean of only 6 animals per day, so the presence of uncontrollable factors such as personnel and weather may have confounded their outcomes.

Some work in this field has employed both physiological and behavioural measurements of stress (Cockram and Corley, 1991; Dunn 1990; Ewbank et al., 1992; Price and Tennessen, 1981; Jarvis et al., 1996; and Tarrant et al., 1992), while others have used only physiological ones (Petty et al., 1994; Mitchell et al., 1988; Wythes et al., 1988a,b). Behavioural observations have sometimes been used only in a general way, without quantification. Ewbank et al. (1992), for instance, used the term "signs of distress (jumping, bellowing, trembling)" when observing the behaviour of cattle as they were handled into a head-restraint inside a stunning pen.

## 2.4 Stress and Fear

Animals can be stressed by either psychological stress, such as handling by humans, electric prodding, noise, and novelty, or by physical stress such as hunger, exhaustion, and thermal extremes. Stress is a dynamic, modular response in an animal to adverse conditions in her environment. The biology of stress involves a complex interaction between physiology and behaviour (Toates, 1995).

Fearfulness is the underlying propensity to be more or less easily frightened, which depends on the genetic, experiential, and nutritional aspects of an animal's life (Jones, 1996). Fear, which is a psychological response to stress, is induced by a perceived or real danger, in which the frightened animal usually attempts one of the three "F's" -- freezing (keeping as quiet and still as possible), flight, or fight, in response to the danger (Gray, 1987). Energy metabolism and cardiovascular adaptations occur which allow the rapid redistribution of blood and energy towards muscles and the brain (Boissy, 1995). Fear is the primary emotional response to an aversive situation such as that found in an abattoir. It is a powerful emotion which effectively competes with and progressively inhibits all other motivational systems, such as feeding, exploring, socializing, and grooming. If the elicitation of fear is sudden, intense, prolonged, or inescapable, it can lead to a failure to cope, which can in turn lead to a rapid breakdown in homeostatic mechanisms in the body (Jones,1996). In this case, all active behavioural responses may cease, and the animal may become very quiet (Fraser & Broom, 1997).

## 2.5 Behavioural Components of Fear

### a) Genetic Influence

Heritable traits such as temperament have been shown to interact with environmental factors such as previous experience with handling. Cattle from excitable genetic lines or breeds (such as Brahman) are more wary of novelty such as is found in an abattoir than cattle with a calmer temperament, and they are more likely to become agitated, balk and back up in a chute (Grandin, 1997c).

### b) Avoidance-Escape Behaviour

Domestic animals still have anti-predator mechanisms, derived from ancient wild ancestors, which become evident under acute stress conditions. Reactive behaviour is sometimes in the form of simple reflexes, such as sudden defecation or urination following an invasion of individual space. Vocalizations can serve to communicate alarm and threat. Visual contact is very important in social group negotiations, and crowded conditions force animals to violate others' space. Avoidance reaction is normally flight if the animal is able to. In cattle, avoidance reactions can be either passive or active, and have characteristic gestures and postures associated with them, such as lowering the head in submission or attempts to escape. Agonistic behaviours, such as butting and mounting, are sometimes induced by avoidance, flight, and submission reactions to real or perceived danger. Cattle use vocalization normally, as a sign of estrus, thirst, hunger, alarm, or pain, or for communication with conspecifics (Fraser and Broom, 1997).

In pre-slaughter handling in an abattoir, Cockram and Corley (1991) observed numerous types of avoidance/escape behaviour in cattle, including struggling, kicking, vocalization, which may be considered “active” responses to fear stress, as well as other behaviour such as slipping, defecating and urinating, which are perhaps more difficult to interpret as either very fearful reactions or simple reflexes. Grandin (1997a) used a standard scoring regime to measure frequencies of cattle balking, falling down, slipping and vocalizing in a knocking box, and suggested that vocalization scoring in particular is perhaps a useful practical indicator of welfare in assessing pre-slaughter stress. The scoring system in the USDA survey she conducted in 1996 used an occurrence of vocalization (in all cattle types) of <0.5% in the stunning box area, as an “excellent” score, and <3% as an “acceptable” score for a slaughter plant.

**c) Novelty**

Animals are strongly motivated to explore and investigate when they are faced with a new environment. This behaviour equips the animal with a system of adjustability which can be used on short notice if necessary for a defense or escape reaction (Fraser & Broom, 1997). Investigative behaviour facilitates learning, in which the animal gathers and processes information about her surroundings. Important determinants of the animal’s ability to cope include the animal’s own control over the situation, her familiarity with the stressor, and her expectations of what might happen to her. The anticipation of an aversive event can itself cause fear.

It is known that novelty is a strong stressor, especially when the animal is suddenly confronted with it and it is perceived as a threat, and will cause balking in cattle if the confrontation is sudden (Grandin, 1997b). If a loud noise is repeated many times over, in a situation where the animal is not directly injured, habituation develops. However, if there is a repeated exposure of very loud noise that directly injures the animal, habituation does not follow (Toates, 1995). Although animals can habituate to non-aversive procedures such as entering a restraint device, they do not habituate to very aversive procedures, whether the procedures are painful or not (e.g., shearing for sheep)(Grandin, 1997b).

**d) Disruption of Social Structure**

The cow is a herd animal who requires a stable social structure to feel secure as part of a cohesive social unit, which ensures survival (Fraser and Broom, 1997). Individuals who form a stable social group may significantly protect each other from stress (Wiepkema, 1987). During the culling process, a cow is removed from familiar herd-mates, familiar people and home environment. Disruption, instability, and uncertainty therefore create the opportunity for fear to preoccupy her life. In a study with dairy heifers, Adeyemo and Heath (1982) found that psychological stress was induced in heifers who were separated from companion heifers known to them, in which they showed signs of distress and high plasma cortisol levels.

**e) Previous Experience**

Cattle tend to stick to a previously-learned safe choice. In a preference test involving switching choices of either walking to the left or right in a Y-maze, walking through or being squeezed in a squeeze-chute, Grandin et al. (1994b) found that cross-bred beef heifers resisted changing a choice once accustomed to walking through on a particular side, even where they showed a preference to avoiding being squeezed in the chute. It was suggested that the more severe the aversive treatment the more likely the animals are to overcome their reluctance to change a previously learned choice.

Prior gentle handling on the home farm may not help an animal adapt to slaughterhouse handling. For instance, in broiler chickens, Kannan and Mench (1996) found that prior gentle handling did not affect the stress response in pre-slaughter handling. On the other hand, previously experienced fear in response to handling may sensitize the animal to similar handling at the slaughterhouse. Grandin (1992) found that cattle who were behaviourally agitated during restraint and handling in one instance were more likely to become stressed the next time they were handled in a similar way. Tameness in animals doesn't necessarily generalize to all procedures, especially if the procedure is highly aversive (Grandin, 1997b).

**f) Mixing and Crowding**

Mixing strange animals together has been shown to cause both psychological and physiological stress. For instance, Price and Tennessen (1981) found that mixing bulls prior to slaughter was the main cause of dark firm dry (DFD) meat in this group of animals, due to a combination of acute psychological trauma and physical exertion. Kondo and Hurnik (1990) found that the greatest number of agonistic encounters happened immediately following re-grouping of lactating dairy cows, and that they diminished from then on. Hasegawa et al. (1997) found that member exchange between groups of first-calf Holstein heifers caused increased aggression, and that those heifers who were moved lost dominance rank and experienced a decrease in milk production.

Crowding has been shown to cause stress in dairy cows (Friend et al., 1979). In this study, 4 groups of cows were restricted to stall-spaces of .75, .63, .5, and .37 free-stalls per cow, and when compared, the total glucocorticoid response was inversely related to space available to the cow.

## 2.6 Physiological Components of Fear

### a) Sympathetic Adrenal Medullary Axis

During the fear response, the sympathetic adrenal medullary (SAM) axis is activated through a direct pathway in which neurons terminate on target organs and release norepinephrine, and/or through an indirect pathway by the adrenal medullary release into the bloodstream of norepinephrine and epinephrine (Toates, 1995). These two catecholamines raise blood pressure, stimulate respiration, dilate respiratory passages, and stimulate the general metabolic activity of cells. They cause vasodilation of blood vessels in skeletal muscles (Judge et al., 1989), and vaso-constriction of vessels to organs not immediately essential, such as the gut. They promote the activity of enzymes that break down glycogen in the liver and muscles (glycogenolysis), and so increase the concentration of glucose in the bloodstream. Epinephrine depletes glycogen and potassium in the muscle (Lawrie, 1985), and its effects are rapid and short-lived; the hormone is inactivated in the liver within three minutes of its release (Curtis, 1968). Norepinephrine is associated with a "fight" response, in which there is an element of control, whereas epinephrine is associated with the "flight" response, in which there is an element of loss of control (Toates, 1995).

High amounts of epinephrine released into the bloodstream, due to acute or chronic anxiety states can cause muscle tremors (Kornegay and Thomson, 1989), among other things. Muscle tremors are involuntary movements in one or more body parts, produced by rhythmic alternating contractions of opposing muscles. Trembling was used (although not quantified) by Ewbank et al. (1992) as in indicator of distress in cattle as they were handled prior to ritual slaughter in a stunning box with or without a head-restraint.

**b) Hypothalamic Pituitary Adrenocortical Axis**

In conjunction with the SAM axis, the hypothalamic-pituitary-adrenocortical (HPA) system stimulates cortisol production in response to "first experience" situations which involve novelty, uncertainty, unpredictability, or lack of control (Toates, 1995). Cortisol, the major corticosteroid in cattle, increases hepatic glycogenolysis and gluconeogenesis, protein catabolism, and thus elevates plasma glucose concentration. It also modulates the release of nor-epinephrine, which protects the body from stress. It is anti-inflammatory and immunosuppressive, and its release influences learning and memory which in turn facilitate exploration of a novel environment (Brown, 1994). Neural input, such as the stress of confrontation with a predator, activates the hypothalamus to produce corticotrophin - releasing factor (CRF), which is carried through a portal system to the pituitary gland. In turn, the pituitary gland produces adrenocorticotrophic hormone (ACTH), which promotes the synthesis and secretion of corticosteroids from adrenal cortex. There are corticosteroid receptors located at various sites throughout the body, and there is feedback inhibition on the HPA by receptors at the pituitary. The binding capacity of these receptors depends, in part, on early experience, such as handling by humans (Toates, 1995). Most of the cortisol in plasma is bound to an alpha-globulin called transcortin, and appears to be inactive in this form, so it is the total reserve of cortisol that is measured in most studies (Tume and Shaw, 1992b).

Baseline cortisol values for lactating dairy cows have been established. Schalm's (Jain, 1986) text stated that a normal cortisol value is 4.7 ng/ml for lactating cows. Alam and Dobson (1986) examined the effect of various veterinary procedures on plasma cortisol in a small sample (n=11) of non-lactating and lactating Holstein cows. Their baseline value was 2 ng/ml, and it increased to a maximum mean value between 6.5±2.5 ng/ml and 13.8±5.6 ng/ml by approximately 13 to 27 minutes after the different procedures, which included rectal palpation, intramuscular injection, jugular venipuncture and jugular vein catheterisation. Mitchell et al. (1988) found a baseline level of 9 ng/ml cortisol in unstressed Friesland and Nguni cows. These cows were accustomed to handling, and the blood samples were collected via indwelling catheters once the animals were used to them.

Handling has been shown to increase cortisol levels in cattle, but not as much in Holstein cows as in beef cattle. Hand-reared cattle may secrete lower concentrations of cortisol than extensively-raised cattle in response to aversive handling. Lay et al. (1992) used tame Holstein cows to test the relative aversiveness of freeze and hot-branding of cattle, and found that restraint in a headgate and fitting with a jugular cannula produced a mean cortisol value of 13 ng/ml. On the other hand, Mitchell et al. (1988) found that cross-bred Brahman

X Hereford X Afrikander steers and heifers showed a mean value of 63 ng/ml cortisol after handling by putting them through a chute into a headgate and taking a blood sample from the jugular vein.

Several studies have measured cortisol at time of slaughter. Mitchell et al. (1988) found that post-stunning plasma levels of cortisol in cattle were 32.0±2.0 ng/ml. Petty et al. (1994) found that *Bos indicus* cattle who were stunned prior to conventional slaughter had an average cortisol level of 43.4±22.7 ng/ml (n=60); Tume and Shaw (1992a) found that beef bullocks killed in a commercial abattoir had an average cortisol level of 44.6±1.9 ng/ml; Ewbank et al. (1992) found that cattle who were stunned in a free-standing position had a mean cortisol concentration of 26.2±9.7 ng/ml versus 55.5±14.2 ng/ml for cattle who were stunned with their heads restrained. Dunn (1990) found a mean cortisol concentration of 48.4±26.3 ng/ml for conventionally slaughtered mature cattle, a mean of 100.7±40.3 ng/ml for those slaughtered in a ritual fashion in an inverted position, and a mean of 55.5±39.6 ng/ml cortisol for those slaughtered in a ritual fashion in an upright position. Cockram and Corley (1991) found an average of 63.4 ng/ml cortisol in conventionally slaughtered beef cattle who were shipped direct from farms as well as from auction markets to an abattoir in Scotland.

Cortisol measurements have been shown to be time-dependent. Lay et al. (1992) found that dairy cows subjected to either freeze or hot-iron branding, had cortisol levels elevated in the period from 5.5 minutes to 25.5 minutes post-branding. Alam and Dobson (1986) found that cortisol levels increased for approximately 13 to 27 minutes after various routine veterinary procedures. Whether cortisol reached peak values was not reported in the studies referred to earlier. Mitchell et al. (1988) collected blood samples after the beef steers and heifers stood in a chute for approximately 15 minutes. Petty et al. (1994) took blood samples 60 s following initiation of a procedure (ritual cutting, or conventional stunning) leading to eventual loss of consciousness, but did not indicate the time the animals spent in the stunning-box itself nor in the lead-up chute before slaughter. Tume and Shaw (1992a) recorded the time between stunning and sticking for all the cattle in their experiment, but did not indicate time intervals in the pre-stunning period, nor if there was any difference in this interval between the two groups that were compared. Ewbank et al. (1992) accounted for the time the cattle spent inside the stunning box (5.6 s and 34.2s respectively for free-standing and restrained positions), and between stunning and sticking, but gave no indication of time spent in the lead-up chute. Dunn (1990) measured the time the animal spent in the stunning-box (mean of 103.8s and 11.1s respectively for inverted and standing positions prior to ritual slaughter), but did not analyze its possible relationship with cortisol concentration. One of these studies did examine the relationship between cortisol and time spent in the chute. Cockram and Corley (1991) measured the time the cattle spent in all locations from lairage pen to stunning pen, and found a positive correlation between cortisol and the time spent in the pre-stun pen (median 1.9 min), total time spent during movement from lairage to stunning (median of 6.6 min), and time spent standing still facing forwards. Interestingly, they also found a negative correlation between cortisol concentration and number of times the animal was hit with a plastic pipe.

There are other problems with reports of cortisol response to stress. Rushen (1991) argued that conflicting results found in the literature, combined with the issues of whether the metabolic consequence of cortisol are related to unbound rather than total corticosteroids, and the high-frequency, low-amplitude pulsatile release of cortisol making it difficult to acquire a representative reading, all contribute to creating doubt about the reliability of cortisol measurements in predicting stress responses.

**c) Packed Cell Volume %**

Differences in hematological values in cattle are due to physiologic differences such as excitement, muscular activity, water balance, ambient temperature, quality of nutrition, age, breed, and possibly sex. Packed cell volume (PCV) % is a measure of the concentration of all the formed elements (cells) of blood, including erythrocytes, leucocytes, and platelets (Campbell, 1993). Normal PCV % for adult cows is 33.6%±5.2, which is lower than for yearling cattle, who have a normal PCV of 35.9%±4.3 (Jain, 1986). Lactating cows were shown to have normal mean PCV values of 25-35% (Kronfield et al., 1982). Increases in environmental temperature tend to increase PCV %. Fear or excitement may cause splenic contractions (as a result of epinephrine release), resulting in larger values of PCV% and concentrations of hemoglobin, and red blood cells (RBCs). Increased heart-rate (also caused by epinephrine release) has a tendency to increase the white blood cell (WBC) count. Also, numbers of blood platelets increase with epinephrine. Blood glucose, lactate, and free fatty acids levels are increased during a fear response, due to the action of the SAM and HPA (Jain, 1986). It has been shown that fed beef cattle who were transported long distances and deprived of water tended to be dehydrated at the time of slaughter. For example, in a study that investigated the influence of pre-slaughter procedures on the behaviour, handling, bruising and dehydration of a mixed population of slaughter cattle ranging from beef steers to cows, Jarvis et al. (1996) found that there was a trend for PCV% to increase with distance traveled from markets. They found a median PCV of 43% for cattle transported a distance <64 km (n=29), 44% for a distance of 64-129 km (n=33), and 48% for a distance > 129 km (n=9).

**d) Creatine Phospho-Kinase**

The power of a muscle fibre largely depends on how fast adenosine tri-phosphate (ATP) can be supplied to operate contractions. Creatine phospho-kinase (CPK), or creatine kinase (CK) as it is often known, is an enzyme involved in the rapid conversion of adenosine di-phosphate (ADP) and inorganic phosphate (Pi) to ATP in skeletal muscles during contraction (McGilvery, 1979), when the animal requires high-energy phosphate in tissues to enable her to exert herself quickly to avoid or escape a perceived danger. It is released into the bloodstream only if some muscle breakdown has occurred, such as from an injury, bruising or from exhaustion, or from starvation, in which an animal is utilizing muscle for fuel to survive. For instance, exhaustion in horses sometimes results in post-exhaustion renal shut-down, indicated by high plasma creatinine, urea, and CPK (Merck, 1991). A baseline normal CPK value (on-farm, pre-transport) for fed steers used by Tarrant et al. (1992) was 34 U/L (n=144). This study examined the effects of stocking density in long-distance transportation of steers on physiology, behaviour and carcass quality. Following transport of these steers the workers found a mean CPK value of 278 U/L, with values increasing with increasing stocking density. The highest stocking density they used for a 24-hour-long transport resulted in an average of 367 U/L (n=24). In another study at an abattoir, Cockram and Corley (1991) found a mean CPK value in exsanguinated blood of beef cattle of 243

U/L.

**e) Effects of Stress on the Carcass**

At death, extracellular glucose is unavailable to the muscle, and the main source of energy for continued glycolysis is muscle glycogen. Glycogen is present in most body cells, but is in the highest concentration in the liver (2-10% wet weight). Skeletal muscles contain between 0.5 - 2.0 % glycogen, and because of their mass, contain the body's largest store of glycogen (Pearson in Price and Schweigert, 1987). Muscle glycogen is catabolized to lactic acid until a pH is reached when the glycolytic enzymes causing the conversion become inactivated. This point is called the ultimate pH (pHu), which is close to the isoelectric point of many muscle proteins. Normal muscle pH in the living animal is 7.0-7.4. By approximately 45 minutes *post mortem*, pH decreases to approximately 6.4-6.5 in healthy, unstressed animals, and ultimate pH is reached at 24 hours *post mortem*, and is approximately 5.5-5.6 (Lawrie, 1985).

If the rate of glycolysis increases due to acute stress, pH falls more quickly *post mortem* as compared with that in unstressed animals. If sufficient stores of glycogen are present in the muscle then the pHu will be a lower value than normal (<5.5) or will be reached sooner than normal. This condition is called pale soft exudative (PSE) meat, a condition that is more common in pigs than in cattle (Lawrie, 1985).

If on the other hand, pre-slaughter stress (including fatigue, hyperactivity, and starvation) leads to muscle glycogen depletion, there is a risk of a higher-than-normal pHu in the muscle. In this case there is the potential of the meat spoiling, due to bacterial growth, and being unacceptably dark in color. This condition is known as dark firm dry (DFD) meat, otherwise known as "dark-cutting" meat. It is a manifestation of metabolic exhaustion, which Gregory (1994) has suggested for use as a welfare indicator. The dark color is due to the high water-binding capacity in cells, which affects the reflection of light (Lawrie, 1985), and the inability of the cells to take up enough oxygen to form a bright red pigment, oxymyoglobin (Pearson in Price and Schweigert, 1987). Glycogen deficiency appears to result from exposure to acute or chronic stress during the pre-slaughter period, and consists of increased energy demand of muscles and adrenergic activation. In an experiment, epinephrine administered to beef cattle before slaughter to induce muscle tremors considerably depleted glycogen reserves, causing a high pHu, and confirmed the view that fear is an important factor in the development of DFD meat (Howard and Lawrie, 1957 in Lawrie, 1985). McVeigh et al. (1979) found that young Friesian bulls exposed to a combination of exercise and emotional stress lost more glycogen than from exercise alone. In a dark-cutting muscle, pH is approximately 6.8 at 45 minutes PM, and 5.8 or higher at 24-hours PM (Lawrie 1985). Although DFD meat is seen as undesirable in fresh meat (poor color and texture, prone to spoilage) it does have certain advantages for processors. It has improved water-binding properties as compared to normal meat, providing good sausage-making properties, better processing yields and improved juiciness (Judge et al., 1989). Commonly, meat with pHu of 6.0 or higher is called DFD meat (Warriss, 1990). Criteria for this condition sometimes include a combination of an appraisal of muscle color and pHu of the *longissimus dorsi* muscle *post mortem*. The incidence of dark-cutting has been a useful indicator of acute pre-slaughter stress, in fed beef cattle. Dark-cutting carcasses of fed beef cattle occur in a small percentage of this population (1-3%), and have been shown to be mainly caused by agonistic behaviour, involving physical exertion, especially mounting of other cattle during the pre-slaughter period. Price and Tennessen (1981) found that regrouping yearling bulls prior to slaughter resulted in agonistic encounters, and was far more important than exposure to a novel environment or loading density in transport, in causing dark-cutting carcasses. Their study found that 73% dark-cutters resulted from regrouping (they used color of meat criteria to quantify DFD condition of carcasses, not pHu measurements). Kenny and Tarrant (1988) found that muscle glycogen loss and pHu increased with the number of times a heifer mounted another during the pre-slaughter period. The fact that this last group were female animals may also have influenced the incidence of DFD conditions. Scanga et al. (1998) showed that heifers were more prone to producing DFD than steers, and speculated that it was due possibly to estrogen secretion.

**f) Effects of Lactation**

In general, non-lactating cows have greater concentrations of red blood cells (RBC), hemoglobin, and PCV % than lactating cows. Corticosteroid responses are influenced significantly by stage of lactation and milk yield, with high-producing and late lactation milk-cows exhibiting a lower level of response (Jain, 1986). This is because concentrations of metabolic hormones are altered to allow mobilization of energy stores during early lactation when energy demand for milk production exceeds dietary intake of energy. Cook (1997) found that cortisol response to stress appeared to differ between lactating and non-lactating sheep who were exposed to an acute stressor (barking dog). Lactating animals had a slightly higher basal level of cortisol, and a lower cortisol response than non-lactating animals. Sartin and Kemppainen (1988) investigated the effect of milk production on cortisol concentrations during a period of negative and positive energy balance, in Holstein cows . They did not find any difference in cortisol response between low and high producing cows, but found that high-producing cows had lower concentrations of cortisol at d 30 (10.4±1.6 ng/ml) than at d 90 postpartum (14.8±1.6 ng/ml) or the non-lactating state (17.8±2.3 ng/ml). They speculated that the purpose of this may be to limit gluconeogenesis from protein stores during early lactation in favor of lipid mobilization and gluconeogenesis in the liver instead.

The stress of early lactation (first ten weeks *post partum*) and negative energy balance during this stage often results in weight loss in a dairy cow. Consequently, a high-producing dairy cow should be in Body Condition Score (BCS) 3.5 and gaining weight at the time of drying off to counteract this loss of weight. High weight loss compromises the reproductive efficiency of cows who are in BCS 2.5 or lower in early lactation (Fredeen, 1990).

**g) Effects of Ill-health, Injury, Exhaustion, and Starvation**

The behaviour of a cull cow during the pre-slaughter period may be affected by disease conditions that may be the cause of the decision to cull her. Disease conditions in lactating dairy cows, such as milk fever, dystocia, retained placenta, metritis, ketosis, and mastitis, also result in loss of weight due to inappetence. Disease is accompanied by symptoms such as listlessness, fatigue, reduced social interaction, loss of appetite, fever, and mental deficits including confusion, learning problems, and impaired memory (Gregory, 1998).

Bruising normally results from injuries associated with transport, mixing with other cattle, and handling at auction markets. Jarvis et al. (1995) found that the incidence of bruising of carcasses was higher in cattle who came through cattle markets compared with those who came directly from farms, and that the use of driving instruments caused bruising. Longer marketing times, cattle marketed through auctions, understocking or overstocking in transport, agonistic encounters of animals unfamiliar with each other, chronic stress, and fasting, all cause animals to be more susceptible to bruising and shrinkage (loss of weight) (Jarvis et al., 1996). When an animal is bruised, the bruised site accumulates exudate antemortem, and can cause an elevation of pH or maintenance of high pH *post mortem* (Gregory, 1996).

A cull dairy cow may become exhausted during the process of culling. A state of exhaustion occurs when glycogen stores become depleted and lactic acid production increases through glycolysis. Lethargy, fatigue, weakness, trembling, stiffness, and pain are associated with exhaustion (Merck, 1991).

## 2.7 Abattoir Management Factors

### a) Design of Abattoir Facilities

Grandin (1979, 1982, 1983, 1984, 1993, 1994a, 1996, 1997a,b,c) has consistently shown that safe and low-stress movement of animals, slaughterhouse facility design, and assessment of welfare problems can be accomplished by using knowledge of natural animal behaviour such as the following behaviour of cattle, and natural vision of pigs. For example, Grandin's (1984) serpentine chute design for herding cattle smoothly in large feedlots and in slaughterhouses, was based on the use of cattle's natural following behaviour.

In a study conducted in 1996, in which Grandin (1997a) surveyed 24 federally-inspected plants in 10 states in the U.S.A., the presence of air blowing down chutes into cattle's faces, slippery floors, the loud hissing noise of air-exhaust valves on pneumatic doors, poor lighting, and the excessive use of electric prodding of livestock were identified as stressors for livestock.

Animals are very fearful of losing their footing (Gray, 1987). The American Meat Institute guidelines for scoring slipping and falling in the stunning chute area, indicate that an incidence of 15% or more animals slipping constitutes a serious problem, and that slipping of less than 3% of the animals is an acceptable rating (Grandin, 1997a).

**b) Electric Prodding and Noise**

Electric prodding, and sudden loud noise, have been identified as stressors in the slaughterhouse. Grandin (1983) indicated that excessive stress was caused by improper use of electric prods (e.g., in the lairage pens vs only in the single file chute), steep ramps and mixing strange animals in slaughterhouses. Cockram and Corley (1991) identified the routine use of driving instruments as stressful to cattle in a slaughterhouse, and recommended using alternative means of handling cattle other than with an electric prod. The USDA (Grandin, 1997a) recommends prod usage to be 5% or less in the stunning-box area for an "Excellent" rating, and 20% or less for an "Acceptable" rating.

As stated earlier, Talling et al. (1996) found that sound exposure in general changed the behaviour of pigs from resting to aroused and attentive. The expression of the response depended on the properties of the sound stimuli, and they concluded that abrupt changes in the sound environment should be eliminated for smooth handling of pigs**.** Warriss et al. (1994) found that there was a positive relationship between sound level in slaughterhouses and indices of poorer meat quality. Agnes et al. (1990) found that both loading and noise have an important role in transport stress. Wythes et al. (1988a) found that noise and disturbance during a resting period before slaughter appeared to be more stressful (higher muscle pH at 24 hours) than mixing of cattle. Orihuela & Solano (1994) found that an increase in noise in the stunning area, as the slaughter process progressed, caused increased agitation in cattle.

**c) Handling Techniques & Duration of Time Spent in the Single File Chute**

Handling practices and the amount of time an animal spends in the chute and stunning-box have been shown to influence the stress she experiences. Smith and Dobson (1990) found that farmed red deer who were handled the least prior to slaughter exhibited the least agitation, their blood contained lower cortisol concentration, and their muscle pH was the lowest (< 5.74) as compared to those who were handled more prior to slaughter. Orihuela and Solano (1994) showed a positive correlation between the order of entry in groups of cattle and time taken to go through a single-file runway in a slaughterhouse, indicating increasing handling problems the longer the animal was exposed to stressors in the chute. They found that last-position cattle took longer to reach the stunning-box, were more difficult to handle, and were prodded with the electric prod more often than cattle at the front of the race.

**d) Training Plant Employees in the Principles of Animal Behaviour**

Apart from improving animal welfare, there are other benefits of gentle handling of animals during the pre-slaughter period. Warriss (1990) suggested that pre-slaughter handling of cattle can affect both carcass and meat quality, and that the natural behaviours of cattle, particularly their following and herding instincts, can be exploited to make handling easier.

Grandin (1996) reported that balking and refusing to move are often caused by basic design mistakes in the chute system, such as poor lighting and high-pitched noises; and by untrained employees, who are more likely to use the electric prod when the animal balks. Grandin (1993) emphasized the importance of employees having a working knowledge of basic principles of animal behaviour, in order to handle animals gently and effectively in the slaughterhouse. Three such principles are flight zone, point of balance, and vision. Flight zone refers to the individual space or distance an animal requires to perform such basic movements as lying down and getting up, and for her personal sense of security, beyond which she may feel threatened and wish to make a rapid exit. If an unfamiliar person stands too close to a cow, and attempts to move her forward, it can be expected that a cow will balk. In relation to humans, the flight zone depends how tame the animal is, whereby a tame animal (such as an average dairy cow) has a smaller flight zone than an extensively raised animal such as a range calf (Fraser and Broom, 1997). A cow's point of balance is her shoulder area, because it is in front of or behind this point that an intruder into her flight zone will cue her to move backwards or forwards in her attempt to get away (Livestock Conservation Institute, 1988). Cattle have approximately 330° panoramic vision due to the location of the eyes on the sides of the head and the horizontal, rectangular pupil, which enables them to detect movement behind them without turning the head. They have a narrow (25-50°) binocular field of vision, which results in low depth perception (Prince, 1970).

Figure 1 summarizes the key factors, referred to in the above review, that contribute to the cull dairy cow's experience of the culling process, from the farm to the abattoir. It forms a conceptual model for use in the assessment of stress and fear during this period.

Figure 1. Conceptual Model for Assessment of Stress and Fear in the Cull Dairy Cow during the Pre-slaughter Period

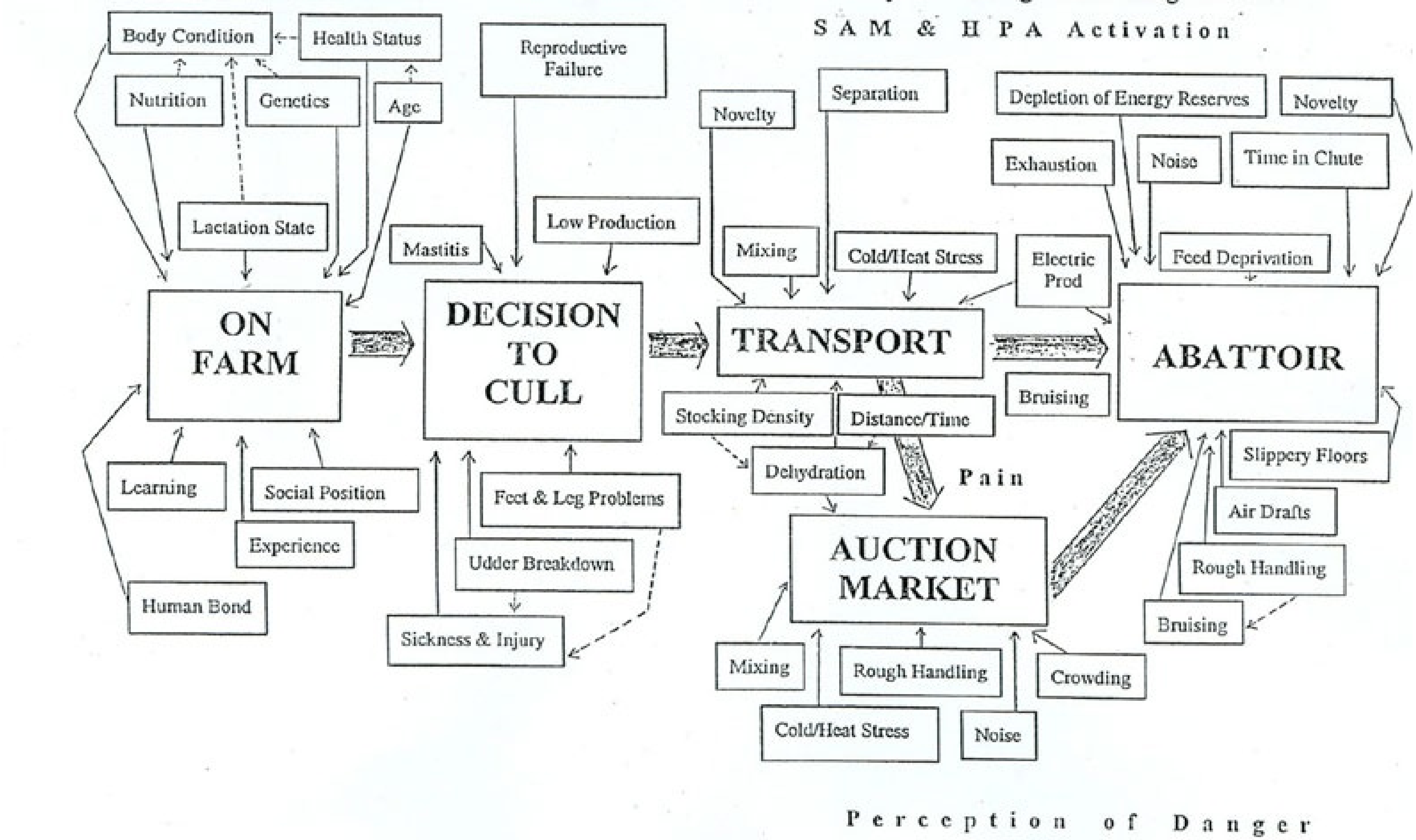



**Figure 1.** Conceptual Model for Assessment of Stress and Fear in the Cull Dairy Cow during the Pre-slaughter Period

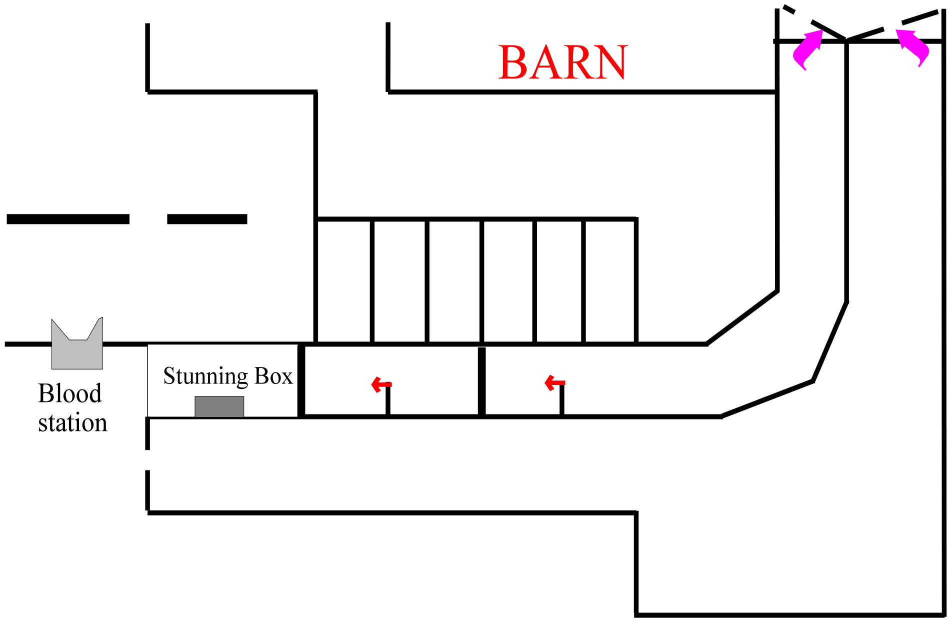



**Figure 2**. Schematic of Single File Chute Area in Abattoir

# Chapter 3. Materials and Methods

## 3.1 Animals

Seven hundred and fifty-two Holstein cull cows were observed as they were handled in the single file chute and stunning-box area ( Figure 2) at HUB Meat Packers, Ltd., located in Moncton, New Brunswick on eight Fridays during the summer months, beginning in mid-June and ending in mid-August, 1998. On these dates, the plant normally killed cows, in addition to fed beef cattle. Beef cows and dairy cows were handled together, being driven from large pens in the barn (18'x40') holding, on average, approximately 25 cows, in small groups of 5 or 6 into the single file chute leading up to the stunning-box. The proportion of dairy cows to beef cows normally handled at the plant was estimated from company records to be 75% dairy/25% beef cows. Of the dairy cows, approximately 90% were Holstein. The cows arrived at the plant the afternoon preceding the day they were killed, and received water ad libitum, but no forage or feed was available to them. The line-speed at this plant at the time was approximately 64 head of cattle per hour, and the average number of cows, both dairy and beef, killed each day was 158.

## 3.2 Treatments

Three sets of conditions were imposed: on each Friday of weeks 1-4, “Control” conditions alternated with “Mufflers”. A workshop on humane handling of cattle was conducted at the plant in week 5. All subsequent data collection corresponded with the staff having been exposed to the “Workshop”. Observation of cows, collection of blood and pH measurements of carcasses resumed on the following two Fridays of weeks 6 and 7. Cows were observed (no blood or pH work was done) in week 8. After a hiatus of three weeks to allow staff to resolve the effects of the workshop, observation of cows as well as blood and pH parameters resumed for a final Friday in week 11.

### 3.3 Noise Reduction

To test the effect of noise reduction on the behavioural and carcass characteristics of cull Holstein cows, mufflers were used on pneumatic doors inside the single file chute and stunning-box on the Fridays of weeks 1 and 3. One muffler was installed by plant maintenance staff on the air-relief valve of a single pneumatic control responsible for raising and lowering two vertical doors in the single file chute, one located at approximately 10 metres from the entrance to the single file chute, and the other located at the entrance to the stunning-box. Another muffler was installed on the air-relief valve of the pneumatic control for the vertically-raised side-door located on one side of the stunning-box, adjacent to the shackling area on the kill-floor. This door was raised following the stunning of the cow in the stunning-box, to permit her body to roll out of the stunning-box onto the floor for shackling of one hind leg and hoisting up to a rail.

A hand-held, portable sound level meter (Realistic®, Cat. No. 33-2050, Tandy Corp., Barrie, Ont.) was used to measure loudness in decibels (dBs). Prior to the installation of the mufflers the air-relief valves produced a sound level of approximately 115 dBs. The sound was a high-pitched, hissing noise. The noise was emitted from the valves each time the vertical doors were raised or lowered, whether for the purpose of separating small groups of cows inside the single file chute, or permitting the rolling or falling of a stunned cow out of the stunning-box onto the shackling floor. Consequently, the noise was irregular in time, but frequent in occurrence. Following the installation of the mufflers, the sound level of the operating valves alone was reduced to approximately 60 dBs, which is that of normal conversation between two humans.

### 3.4 Workshop on Humane Handling of Cattle

To test the effect of a workshop on humane handling of cattle on the behavioural and carcass characteristics of cull Holstein cows, fourteen employees of the plant attended a two-hour workshop, held in the Boardroom of the company offices, on a day of the week on which there was no kill-operation. The employees included 3 barn staff, 3 stunners, 3 shacklers, three foremen, one supervisor, and the livestock operations manager.

The workshop was designed and co-conducted by my advisor Dr. Tarjei Tennessen and myself. The design was based on material made available by the Canadian Food Inspection Agency (CFIA), on guidelines and diagrams produced by Temple Grandin (1993) and the Livestock Conservation Institute (1988a), and according to Temple Grandin's (1979, 1982, 1983, 1984, 1993, 1994a, 1996, 1997a,b,c, 1998) extensive work in improving animal movement at slaughter plants. We instructed staff who herded cows to position themselves behind the cow's shoulder, in her clear scope of vision, in order to move her forward effectively. We recommended the elimination of shadows along the chute by evening out the lighting within it, and also recommended that the same tone of color be used on the walls of the chute and vicinity, so as to reduce distractions for the cows and so improve the handling time in the chute area. We sought to combine four main learning elements in the workshop: visual learning (using an instructional video on herding cattle called Cattle Transportation, written by Temple Grandin and produced by the Livestock Conservation Institute (1988b); some lecturing on principles of animal behaviour in handling cattle; participation on the part of the employees in identifying problem areas and discussing possible solutions; and finally a practical demonstration in the barn, in the use of the hollow cane, which was presented to the staff as an experimental prototype of an alternative tool for the electric prod.

Topics covered in the workshop were organized into four main areas. Principles of cow behaviour included emotions and their functions, cow vision and hearing, flight distance, point of balance, movement, and following patterns. Differences between dairy cows and fed beef cattle were described, covering typical life on the farm, disruption of normal behaviour by the culling process, and the advantages and disadvantages of handling older cattle who are accustomed to people. For instance, it was pointed out that although cull dairy cows may be perceived to move more slowly than fed beef cattle and to require more patience, they are less likely to kick a person who is handling them. Handler participation in reducing the use of the electric prod was the third workshop topic. We referred to the USDA guidelines for electric prod usage (Grandin, 1997a), and introduced the use of a brightly-coloured hollow plastic cane I designed as an alternative to the electric prod. Several of these hollow canes were made available to the staff, and they were encouraged to use them in place of the electric prod. A demonstration in their use was conducted at the end of the workshop, in the barn. The fourth and final main area covered in the workshop was facility design and its role in improving handling efficiency and welfare of cattle. Potential problems that were high-lighted included slippery floors, poor lighting, high-pitched loud noises, and distractions in the chute. Employees participated most actively in this portion of the workshop. Finally, posters entitled “Five Working Principles of Humane Handling of Livestock” (Figure 3) were distributed for posting on walls in the barn and chute areas .

One week after the workshop was conducted, the following measures were in place: lighting intensity was increased in the corner of the single file chute, in front of the first pneumatic door; the walls and storage cabinet beside the single file chute were painted with one grey colour; the drain grates, located in front of each gate from the lairage pens to the adjacent main aisle-way leading to the single file chute, were kept covered over with sawdust and wood shavings to help reduce their slippery surface; several posters reminding personnel of the “5 Working Principles of Humane Handling of Livestock” were posted in the barn and chute areas; and some personnel were using the hollow canes to move the cattle some of the time. At this time, personnel were given several hollow plastic canes with 14" strands of plastic attached on one end, for use in the single file chute and stunning-box area.

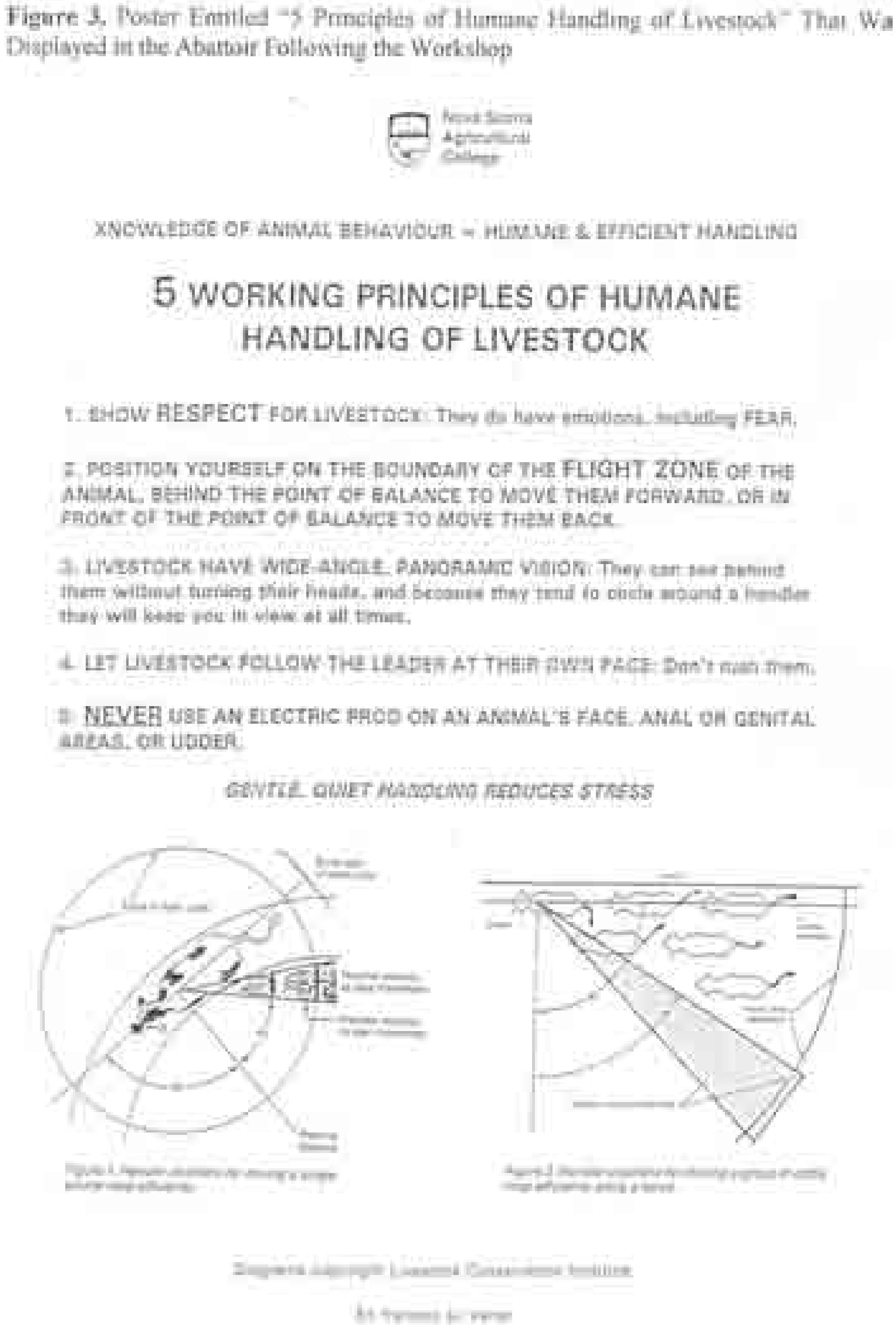



**Figure 3.** Poster Entitled "5 Principles of Humane Handling of Livestock" That Was Displayed in the Abattoir Following the Workshop

## 3.5 Behavioural Observation of Cows

Criteria for evaluating the fearfulness of cull Holstein cows in the pre-stunning period were based on a combination of behavioural and physiological indicators. The behaviour of each cow was measured using focal animal, one-zero sampling. As each animal entered the stunning-box area it was recorded whether or not certain behaviours occurred. Behaviours selected for recording were thought to be indicative of fearfulness or agitation.

### a) Ethogram: Definitions of Behaviour Categories

**Balking** was defined as coming to an abrupt halt as the cow was walking forward, and included backing up in the stunning-box (modified from Ewbank et al.1992; Grandin 1992).

**Struggling** was defined as the rapid movement of two or more legs without a change in the position of the cow, and included slipping (modified from Cockram and Corley 1991).

**Turning** was defined as the rotation of the head and neck to a point past 90 degrees from centre toward either side of the body, as in a first motion to turn the body around to reverse direction. This could involve only the head and neck, or the head, neck, and front part of the body (modified from Cockram and Corley 1991).

**Trembling** was defined as a tremor, or very fine jerky contractions of muscle or of some of the fibres of muscle anywhere on the body which was clearly visible from a distance of 1 metre (modified from Ewbank et al.. 1992).

**Vocalizing** was defined as any utterance of vocal sound emitted from the mouth of the cow which could be heard above the background noise of the stunning-box area, from a distance of at least 1 metre from the cow (modified from Lay et al. 1992; Ewbank et al. 1992; and Grandin 1997a).

Figure 4 illustrates each of the aforementioned behaviours, and Figure 5 illustrates all five behaviours as they appear in the single file chute.

**Figure 4.** Illustrations of Cull Holstein Cow Balking, Struggling, Attempting to Turn Around in the Single File Chute, Trembling and Vocalizing

**Figure 4.** Illustrations of Cull Holstein Cow Balking, Struggling, Attempting to Turn Around in the Single File Chute, Trembling and Vocalizing

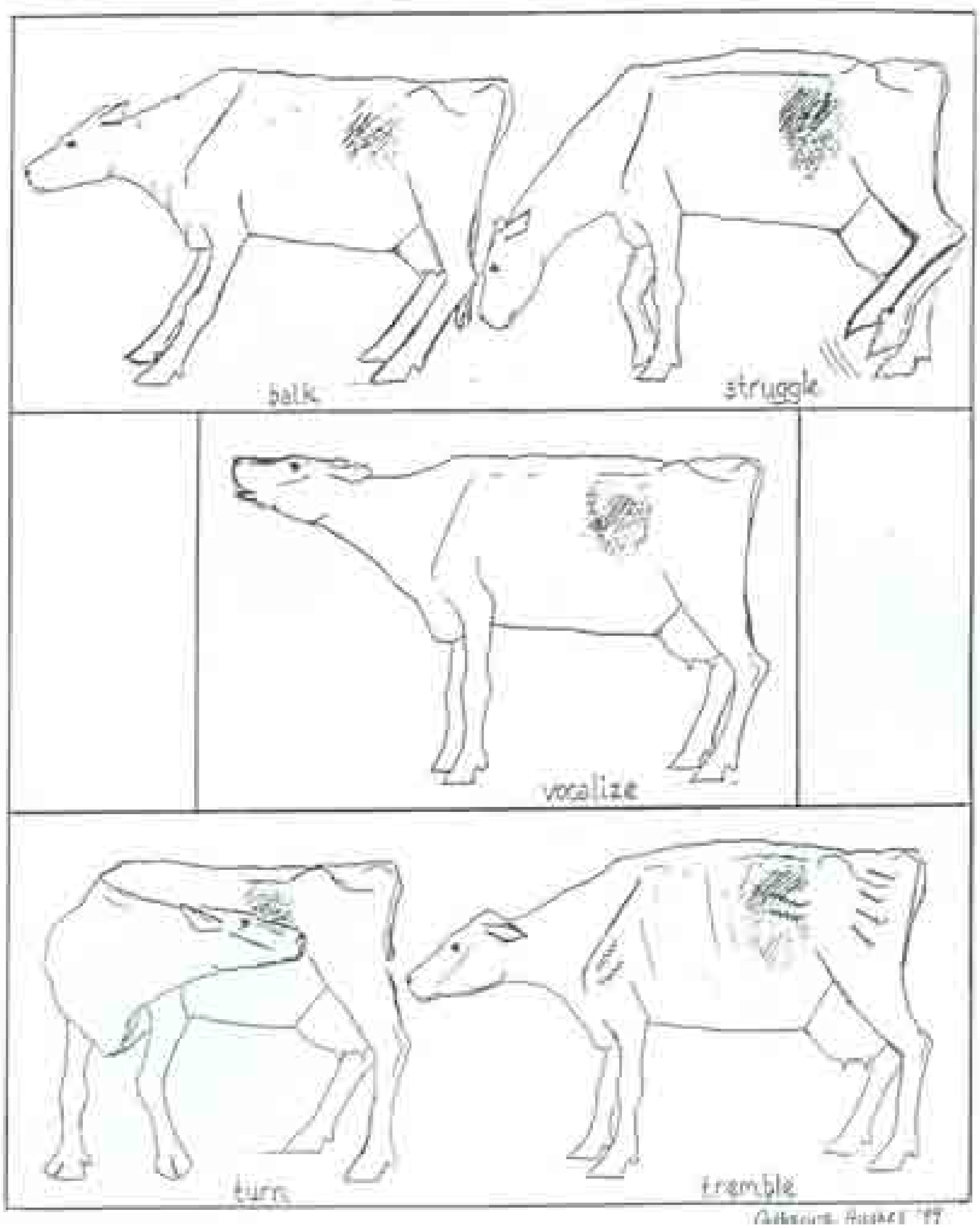
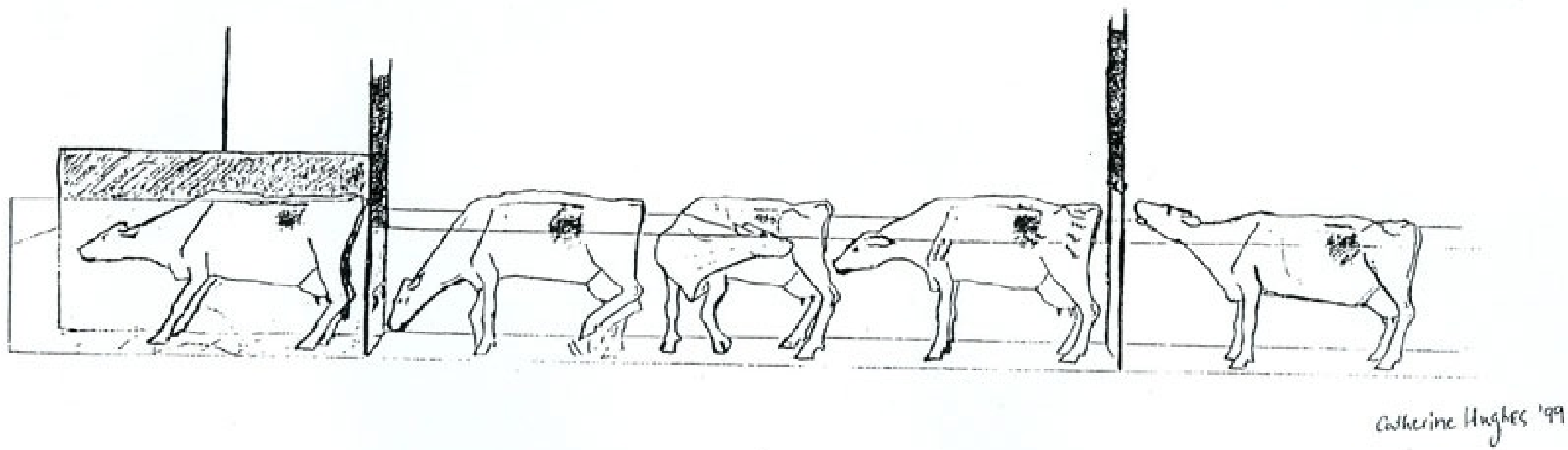


**Figure 5.** Illustration of 5 Behaviours Indicative of Fear in Cull Holstein Cows in Single File Chute at an Abattoir

**Figure 5.** Illustration of 5 Behaviours Indicative of Fear in Cull Holstein Cows in Single File Chute at an Abattoir



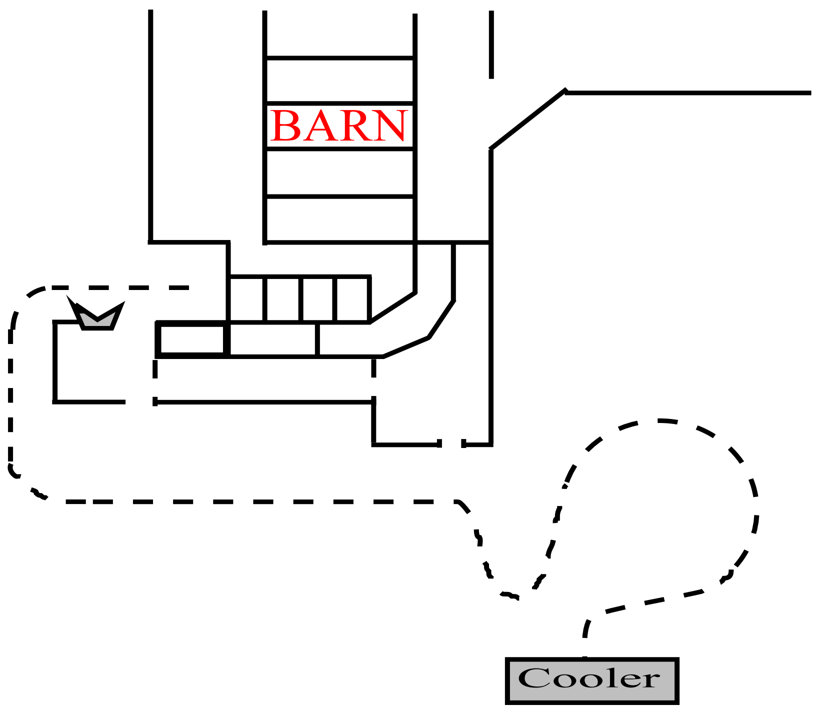



**Figure 6.** Schematic of Barn, Single File Chute and Kill Floor at the Abattoir

**b) Method of Behavioural Observation**

On the morning of the kill, one observer was positioned approximately 4.5 metres from the entrance to the single file chute (Figure 6), and took note of the time of entry into the chute of the first and last cow of each small group, the breed, approximate age ("mature" or "young") of each cow. This observer also measured environmental data just prior to the start-up of the day's operation, and again after the cows were all killed. This information included the temperature and relative humidity outdoors and inside the single file chute area, noise levels in the barn and peak noise level (dB) at the entrance to the stunning-box, and light (Lux) levels (measured with an Optikon Luxmeter, Model EC1, Sweden) at three points inside the single file chute.

At the end of the single file chute (total length was 17.4 metres), 1 metre across from the vertical, pneumatically-lifting door just at the entrance to the stunning-box (dimensions 0.8 x 2.4 metres), a second observer used a standard form to take note of each cow's behaviour and other information immediately prior to her being stunned with a captive bolt pistol in the stunning-box. The occurrence or non-occurrence of the 5 specific behaviours described above (whether she balked, struggled, attempted to turn around, trembled, or vocalized at least once) was noted. This observer also noted the breed, assigned a Body Condition Score of between 1 and 5 (according to Edmonson et al., 1989) , ear-tag number, and exact time of stun. The average duration of time between two cows being stunned was 61 seconds, so this was the interval during which each cow's behaviour was observed. As the cow's body was rolled out of the side of the stunning-box onto the shackling floor following stunning, the observer also noted whether the cow was lactating or not. An udder that was flaccid was deemed non-lactating, and any udder that had turgidity in most or all four quarters, and the characteristic fullness of functional glands, was deemed to be lactating. In the rare case where there was any doubt, the cow was deemed to be non-lactating.

Every cow was observed in this way, whether a dairy cow or beef cow, and later the ear-tag numbers were cross-checked with the plant kill-sheet, the first observer's data-sheet, and that of the blood technician.

### 3.6 Collection of Blood

Physiological criteria used to evaluate the relative fearfulness of the cows in the pre-stunning period included three blood parameters (packed cell volume (PCV) %, concentrations of plasma cortisol, and plasma creatine phospho-kinase (CPK)), and the pH of the *longissimus dorsi* muscle of the carcasses *post mortem*. Packed cell volume % was used to measure hydration status, and to indicate activation of the sympathetic adrenocortical axis causing splenic contractions. Cortisol was used to indicate activation of the HPA system. Creatine phospho-kinase was used to estimate muscle damage due to physical stress such as bruising. Muscle pH was used to indicate acute and/or chronic psychological and physical stress on the cows in the pre-slaughter period.

A 25 ml sample of blood was collected in a small paper cup by a plant employee immediately at the point of exsanguination (following the cutting of both carotid artery and jugular vein) of each insensible cow on the rail, and then handed to a research team technician from the Nova Scotia Agricultural College (NSAC), who filled a labelled, 10 ml, heparinized plastic test tube and put it in a rack. Just prior to receiving the cup of blood, the cow's ear tag number was called out by the plant employee “sticking” the cow, and this number was noted alongside the test tube number; and the exact time of exsanguination (the point at which both artery and vein had been cut, and blood just began to flow freely) was noted alongside the test tube number on the data sheet. Heparin (Hepalean® anticoagulant, 10,000 USP Units/ml, Organon Teknika, Toronto, Ont.) was added to the test tubes in liquid form the previous afternoon, at a concentration of 200 ì l/10 ml tube. Once filled, each rack of sixty tubes was then immediately stored in a cooler containing freezer packs. Another rack of empty tubes was then taken out of the cooler, and placed on a shelf near the technician. The racks were kept on ice for up to seven hours after collection. Upon arrival back at the College lab, these blood samples were individually mixed at room temperature and a hematocrit was taken from each one to obtain a packed cell volume %. Blood was drawn up into a micro-hematocrit tube, spun in an hematocrit centrifuge (International Equipment Co.(IEC) Model CL, Needham Hts, MA) for 15 minutes, and the hematocrit was read using an IEC Micro-Capillary Reader (Needham Hts, MA). The remaining blood in the test tubes was then centrifuged at 1500 g at 4°C for twenty minutes, using a refrigerated centrifuge (IEC Centra-7R, Needham Hts, MA). The plasma (or serum in a few cases) was then pipetted into duplicate micro-centrifuge tubes and stored in a -80°C freezer until later analysis for cortisol and creatine phospho-kinase (CPK) content. Plasma taken from blood collected on the first three experimental days of the study was initially stored in either a -20°C or -45°C freezer, and then moved to the -80°C freezer when space became available. Plasma from the subsequent five experimental days of the study was stored immediately upon packaging in the lab at -80° C.

**a) Analysis of Plasma for Cortisol**

Seventy plasma samples were randomly selected from samples collected for each treatment group, using the Randint Program (Firth, Truro, N.S.). In addition to these samples, 10 samples from healthy, lactating Holstein cows at the NSAC farm in Bible Hill, Nova Scotia, were selected. Plasma cortisol levels were determined by using a solid-phase radio-immunoassay (RIA). A Coat-A-Count® kit (Diagnostic Products Corporation, Los Angeles, CA) was used, and the analysis was conducted by the Diagnostic Laboratory at the Atlantic Veterinary College in Charlottetown, Prince Edward Island. All 220 plasma samples were analysed on the same day.

The plasma samples were analysed approximately 7 months after blood was collected at the abattoir.

**b) Analysis of Plasma for Creatine Phospho-Kinase**

The same plasma samples that were randomly selected for cortisol analysis were also analysed for creatine phospho-kinase content. The analysis was conducted at the Atlantic Veterinary College's Diagnostic Laboratory on the same day as the cortisol samples were analysed, using the "optimized standard method" with a BM/Hitachi 704/705/911 Creatine Kinase UV test (Boehringer Mannheim, Laval, Que).

### 3.7 Examination of Carcasses for Muscle pH

#### a) Equipment

A portable, digital pH meter (Fisher pH/temp Meter 119, Fisher Scientific, Nepean, Ont.), fitted with a spear-type pH probe (Mettler-Toledo International Inc., Greifensee, Switzerland), and a temperature probe (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) was used for all the pH measurements taken at the plant. For the first measurement, at approximately 45 minutes *post mortem* (pH45), the pH meter was re-calibrated during the coffee break at approximately half-way through the morning's kill (8 am - 12 noon). The calibration solutions were at room temperature. For the pHu measurements, conducted at approximately 72 hours *post mortem*, the pH meter was re-calibrated at the end of every rail of approximately 23 paired carcasses. The calibration solutions were stored in the outside pocket of the technician's lab jacket pocket, and so they cooled gradually over the period of time spent in the cooler. Twice during the course of the study, the portable meter was checked for accuracy against a stationary pH meter (Accumet Model 10 pH meter, Fisher Scientific, Nepean, Ont.) in the Environmental Sciences Department of NSAC. The calibration solutions of pH4 and pH7 were also checked for concentration accuracy against fresh pH4 and pH7 solutions at this time, and were refreshed once during the study. On both occasions that the portable meter was checked, it performed to within 0.01 to 0.03 pH units of the stationary unit.

#### b) Method Used to Measure Muscle pH

At approximately 45 minutes and again at 72 hours *post mortem*, two research team technicians positioned along the rail at the appropriate point, measured the pH of the *longissimus dorsi* muscle of one side of each cow's carcass. The temperature of the muscle was also noted at this time. The pH meter was used following an incision of approximately 2 cm into the *longissimus dorsi* (LD) muscle, between the 7th and 8th rib, where there was sufficient muscle mass to make an incision. In most studies of beef cattle, an incision is made between the 10th and 11th ribs, however this was not possible in the case of these cull cows, as they were very thin animals and there was insufficient muscle mass at this location on the carcass. Temperature and pH were noted, along with the ear tag or bar-code contained on the card pinned onto each carcass side, and the exact time the incision was made.

### 3.8 Pregnancy Examination and Collection of Follow-up Data

Examination of the cows for pregnancy was conducted *post mortem* at the evisceration table on one day of each treatment. The examinations were performed by Canadian Food Inspection Agency (CFIA) personnel on two occasions, and by a qualified technician from the NSAC on the third occasion. In each case, all the cows were examined, and only those which had a fetus large enough to detect easily (approximately 8 weeks gestation) were recorded by ear-tag number on a data sheet. Ear-tag numbers of non-pregnant cows were not recorded.

Follow-up data on the cows, including the geographic origin of each cow, the net dressed weight of each carcass, a list of condemned carcasses, the identity of cows who were held for veterinary antemortem inspection, and date of arrival of each cow, were made available by the plant management. Cross-checks were conducted to confirm ear-tag numbers, and order in the chute, from the data sheets of individual research technicians and the plant records. Data from Holstein cows only was selected from the complete data set, and any

Holstein cows who were downers, condemned, held for veterinary inspection, arrived the same day as the kill, or herded from an outside yard into the barn prior to the kill, were disqualified from the study. Only Holstein cows who arrived the day before the kill, were housed in the barn pens, and passed government veterinary inspection were used in the study.

## 3.9 Statistical Analysis

### a) Hypothesis

The hypothesis of the study was that a reduction in the sudden, loud noise produced by air-exhaust valves on pneumatic doors in the single file chute at an abattoir, and training of employees in the humane handling of cattle, would result in observable differences (relative to the status quo) in the fear behaviour of cull Holstein cows at the point of slaughter, thus improving their welfare at this critical time.

Ho: Control Treatment = Mufflers Treatment = Workshop Treatment

Ha: Control Treatment ≠ Mufflers Treatment ≠ Workshop Treatment

### b) Procedures

The study was conducted in a commercial setting, and consequently it was not possible to assign subjects randomly among treatments. Hence, there were potentially confounding variables, as well as many variables not under any research control. The statistical comparisons were used mainly to examine possible indications of effects and trends among the variables. Thus, probability values less than 0.10 were considered significant. All procedures were carried out using the Statistical Analysis System (SAS Institute Inc., Cary, North Carolina).

To examine treatment effects, Chi-square analysis (proc freq) was used to test for differences in frequencies of balking, struggling, turning, trembling, and vocalizing. One-way analysis of variance (General Linear Model, proc glm) procedures were used to examine variation among treatments in PCV%, cortisol, CPK, pH 45, and pHu. Where significant differences in physiological responses were found among groups, Duncan's Multiple Range Test was used to determine which treatments were significantly different from each other.

For the purposes of an exploratory examination of factors in addition to treatment that influenced the responses, explanatory models were constructed using the following procedures. Logistic regression is used to fit a model to a binary response variable such as the occurrence or non-occurrence of a specific behaviour. Hence, logistic regression analysis (proc genmod) was used to test the effect of all known factors affecting each of the five behavioural responses, and rudimentary models were constructed using the following formula:

$$\text{(Logit behaviour)}\log (P \div 1-P) = \mu + \text{Treatment} + \text{Other Factors} + \text{Error}$$

(where P = Probability of the behaviour occurring). Multi-way analysis of variance (proc glm) was used to partition variation among all known factors on the five physiological responses. For all physiological responses, a general linear model was constructed using the following formula:

ã = ì + Treatment + Other Factors + Error

(where ã= individual value of response, and ì = population mean for the response). Interactions were not included in any of the models. Because of missing values, all factors were included in the model one at a time, and if significant combined with other significant factors and treatment. The final models reported therefore, were arrived at by a combination of forward-screening and backward-elimination, to attempt to explain significant effects on the ten fear responses selected for my study. Correlational analysis, to determine both Pearson and Spearman coefficients where appropriate, was also used to investigate associations among all variables recorded.

# Chapter 4. Results

A group comparison of the Control, Mufflers, and Workshop Treatments was conducted, to test treatment effect only. As my analysis proceeded, I ventured slightly beyond these original objectives because I wanted to understand a little more about the cows' experience and what factors may have influenced it.

The following exploratory analysis was not done for the purpose of making formal inferences about responses, but rather as an attempt at gaining insights into the cull Holstein cow's experience during the final sixty-one seconds of her life. Rudimentary models were constructed for main effects only and no interactions were included.

## 4.1 Descriptive Features of Cull Holstein Cows

Independent of the experiment conducted, some preliminary information regarding the composition of a sample of cull Holsteins at an abattoir was derived. Eighty-one point nine percent (81.9 %) of the sample population was mature in age (multiparous), 82.4% were lactating, and 15.6% were pregnant at the time of slaughter (Table 1). As shown in Table 2, 41.4% of the cows were emaciated (B.C.S.=1), the next largest group (32.1%) was skinny but not emaciated (B.C.S.=2), and 26.5% of cows were in good physical condition at the time of slaughter (B.C.S.=3,4,&5). The largest group of cows (31.8%) in the study originated from the province of Nova Scotia (but may have included some cows from Newfoundland which were sold at auction in Truro, N.S.). The next largest group (24.5%) originated from the State of Maine, U.S.A. Cows originating from Prince Edward Island comprised 17.2%, 13.7%

originated from New Brunswick, and the origin of 12.7% of the cows was unknown (Table 3). Average dressed (net) weight of the cows' carcasses was 571.7 lbs (260 kg) (Table 4).

## 4.2 Treatment Effect

The original hypothesis was that reducing sudden loud noise and educating staff in humane handling of cattle would result in a reduction in the fear experienced by cull Holstein cows at an abattoir.

Ho: Control Treatment = Mufflers Treatment = Workshop Treatment

Ha: Control Treatment ≠ Mufflers Treatment ≠ Workshop Treatment

As shown in Tables 5 and 6, two behavioural responses (balking and turning), and three physiological responses (PCV%, CPK, and pH 45) were significantly different in the Workshop Treatment as compared to the Control and Mufflers Treatments. Therefore, it can be concluded that the null hypothesis can be rejected, and it can therefore be stated that there was a significant difference found as a result of treatment effect.

### a) Behavioural Responses

As shown in Table 56, the proportion of cows who **balked** in Control and Mufflers Treatments was reduced by 14.2% in the Workshop Treatment (average 62.5% reduced to 53.6%). Frequency of **struggling** appears not to have been significantly affected by treatment. The proportion of cows **attempting to turn around** in the stunning-box area just prior to stunning in Control Treatment and Mufflers Treatment was reduced by 22.5% in the Workshop Treatment (average 32.9% reduced to 25.5%). Frequencies of **trembling** and **vocalizing** appear not to have been significantly affected by treatment.

**b) Physiological Responses**

As shown in Table 5, there was a small decrease in **PCV%** in Workshop Treatment as compared to Mufflers Treatment (34.9±% vs 36.7±%). There was no significant difference in **cortisol** levels across treatments. There was a significant difference in plasma CPK content, in which cows in the Workshop Treatment showed almost double the content (960 U/L±126.3) as those in the Control and Mufflers Treatments (average 546 U/L±70.3). There was an increase in **pH 45** in the Workshop Treatment (6.82±0.01), as compared to the Control and Mufflers Treatments (average 6.73±0.02). There was no significant difference in **pHu** of the *longissimus dorsi* muscle in the cows' carcasses, among the treatments.

**4.3 Exploratory Analysis:** Effect of Treatment and Other Factors

Factors related to treatment that were recorded included the cattle-handling performance of the individual person working as the stunner of the cow (whether he used the electric prod or not), electric prod usage, and alternative prod usage (Workshop Treatment only). The effects of these factors are reported, and included in the Treatment effect component of the model, where applicable.

Uncontrollable factors, both intrinsic to the cow and management-related, some of which held potential as confounders in the experiment, included origin of the cow, age, body condition, lactation state, pregnancy state, and the order the cow entered the single file chute in the small group of cows herded into the single file chute (SFC) from the larger lairage pens in the adjacent barn. Only the factors showing significant effect were included in models constructed for specific behaviours and physiological responses are reported below. True confounders for a given response are indicated by **[C]**.

**a) Behavioural Responses**

**i. Balking**

Model: log P÷1-P Balking = μ + Treatment + Error

The proportion of cows who balked in the Control and Mufflers Treatments was reduced in the Workshop Treatment (Table 6). There was a significant association between electric prod use and an increase in the frequency of balking (P=0.012, n=752). Balking occurred in 60.5% of the cows who were prodded with the electric prod, as compared to balking occurring in 48.5% of the cows who were moved forward without the use of the electric prod. Singling out the Workshop Treatment (n=343), it was found that the type of prod used tended to affect frequency of balking (P=0.075). Within that treatment, no electric prod used resulted in 36% balking (n=22), the alternative prod (hollow cane) resulted in 48% balking (n=87), and the electric prod resulted in the highest at 58% (n=234).

**ii. Struggling**

As shown in Table 6, frequency of struggling was not significantly affected by treatment, remained quite constant over all the treatments, and seemed unaffected by any of the intrinsic or management factors accounted for in the study.

**iii. Attempting to Turn Around in the Chute**

Model: log P÷1-P Turning = μ + Treatment + Order of Entry + Origin + Error

After the humane handling workshop, the proportion of cows attempting to turn around in the stunning-box area just prior to stunning was reduced from average 32.9% to 25.5% (Table 6). The frequency of turning also appeared to have been influenced by origin **[C]** of the cow, and cows from P.E.I. were more likely to attempt to turn in the stunning-box area (39.2% in cows from P.E.I. vs avg 28.1% in cows from other parts of the region, P=0.032, n=656). It appears that increasing order of entry into the single file chute may have been associated with increasing frequency of turning in the stunning-box area (21.1% of cows entering the SFC in the first position attempted to turn vs 37.8% of cows entering the SFC in the last position, P=0.031, n=675). The time spent in the chute was significantly reduced by the Workshop Treatment (Table 4).

**iv. Trembling**

Model: log P÷1-P Trembling = μ + Treatment + Age + Error

As shown in Table 6, frequency of trembling remained quite constant over all the treatments, and was not found to be significantly influenced by any of the factors accounted for in this study, including treatment effect.

However, there was a slight indication that, over all treatments, **age [C]** tended to affect frequency of trembling. Thirty-seven point two (37.2) % of mature cows were trembling visibly in the area of the stunning-box, while 29.4% of young cows did so (P=0.107, n=656).

**v. Vocalizing**

Model: log P÷1-P Vocalizing = μ + Treatment + Origin + Error

Although frequency of vocalizing was not significantly affected by treatment, there were two factors which appear to have significantly contributed to it; use of the electric prod (8.2% vs 3.9%, P=0.086, n=752); and origin of the cow **[C]** appears to have significantly affected the frequency of vocalizing. A greater proportion of cows from P.E.I. vocalized than cows who originated from other parts of the region (13.9% vs avg 6.5%, P=0.026, n=656).

**b) Physiological Responses**

**i. Packed Cell Volume %**

Model: PCV% = $\mu$ + Treatment + Origin + BCS + Age + Dressed Weight + Error

df=554, R-Sq=0.21, P<0.0001

As shown in Table, there was a significant decrease in PCV% in the Workshop Treatment (34.9%) as compared to Mufflers Treatment (36.7%). Packed cell volume % appears to have been affected by the **origin [C]** of the cow in Mufflers and Workshop Treatment (Table 7). Cull cows from the state of Maine had a lower PCV% (33.9%) than cows originating from other areas (avg 36.4%). It appears also to have been affected by body condition **[C]** in every treatment group. Emaciated cows (BCS=1) had a lower PCV % than cows with more body condition. It also may have been significantly affected by age **[C]** of the cow. Over all treatments, mature cows had a lower PCV% than young cows (35.5%± 0.26 vs.37.3±0.48%).

It appears that approximately 21% of the variation in PCV% may be explained by this model. Most of the variation in PCV%, however, is due to unknown factors.

**ii. Cortisol**

Model: Cortisol = $\mu$ + Treatment + Origin + Order in Chute + Error

df=192, R-Sq=0.13, P=0.0022

Although cortisol level was not significantly affected by treatment in general, there was one treatment-related factor which appears to have significantly (P<0.0008, n=173) contributed to it. There was a weak positive correlation (+0.25) between time in the chute and cortisol level. It appears also that cortisol was affected by origin **[C]** of the cow. As shown in Table 7, cows from Maine and P.E.I. had lower cortisol levels than cows from New Brunswick. The order of entry into the single file chute in the small group may have affected cortisol levels. Cows in the first position entering the chute had lower cortisol levels (17.7±1.4 ng/ml) than cows entering in the last position (27.3±2.3 ng/ml).

Approximately 13% of the variability in cortisol may be explained by this model. Most of the variability of cortisol, however, is explained by unknown factors in this context.

**iii. Creatine Phospho-Kinase**

Model: CPK = μ + Treatment + Error

df=172, R-Sq=0.11, P=0.0018

There was a significant difference in plasma CPK content, in which cows in the Workshop Treatment showed almost double the content as compared to those in the Control and Mufflers Treatments (Table 5).

Approximately 6 % of the variability in CPK may be explained by the model. Most of the variability, however, was due to unknown factors in this context.

**iv. pH of *longissimus dorsi* Muscle at 45 min post mortem**

Model: pH 45 min = μ + Treatment + Origin + BCS + Dressed Weight + Error

df=469, R-Sq=0.15, P<0.0001

There was an increase in pH 45, in the Workshop Treatment (6.82±0.01), as compared to the other treatments (average 6.73±0.02, Table 5). It appears to have been significantly (P=0.0228, n=534) affected by electric prod use on the cows, with a pH value of 6.82 when the electric prod was not used, as compared with a pH value of 6.76 when it was.

As shown in Table 8, pH 45 may have been affected by the origin [C] of the cow over all treatments. Cows originating from Maine and P.E.I. had higher average pH 45 than did cows originating from Nova Scotia and New Brunswick. Muscle pH 45 also appears to have been affected by body condition [C] in Mufflers and Workshop Treatments. As shown in Table 8, over all treatments, emaciated cows (BCS=1) had higher pH 45 than did cows in Body Condition Score 3.

Approximately 15% of the variation in pH of LD muscle at 45 minutes post mortem may be explained by the model. Most of the variability in pH 45 min, however, was due to unknown factors in this context.

**v. Ultimate pH**

$$pHu = \mu + \text{Treatment} + \text{Origin} + \text{Order in Chute} + \text{BCS} + \text{Dressed Weight} + \text{Error}$$

df=428, R-Sq=0.24, $P<0.0001$

Although no treatment effect was found for pHu , an attempt was made to explain some of the variation in pHu according to other factors that were recorded in the study.

As shown in Table 8, pHu may have been affected by origin [C] of the cow. Cull cows originating from the state of Maine had the highest average pHu as compared with cows originating from other areas of the region. Body condition [C] of the cow appears to have significantly affected pHu. Emaciated cows (BCS=1) had higher pHu (6.27±0.02) than did cows with body condition scores >1 (6.07±0.03, Table 8). There was a significant ($P<0.0001$) negative correlation (-0.35) between body condition score and pHu . Order of entry into the chute in the small group showed a tendency to affect pHu (Table 9). Cows coming into the single file chute in the fifth or higher position in the small groups which were herded from the larger pens in the barn, had higher pHu (6.21) in their carcasses than those cows in the third and fourth position (6.11 and 6.10).

Approximately 23% of the variation in pH Ultimate may be explained by the model. Other unknown factors, however, explain most of the variability in pHu.

## 4.4 Practical Improvements at the Abattoir

### a) Time Cow Spent in Single File Chute

Average time that the cow spent in the single file chute was significantly reduced in Workshop Treatment (4.8±0.1 min) over the Control and Mufflers Treatments (6.0±0.2 min, Table 4).

### b) Noise and Light Levels in Chute

As shown in Table 10, the mean peak noise level at the stunning-box was not reduced substantially by the installation of mufflers on the air-exhaust valves of the pneumatically-operated vertical doors in the single file chute. However, the sudden loud noise of these valves alone was reduced substantially as a result of the addition of the mufflers, from approximately 115 dB to approximately 60 dB. Light levels in two locations in the single file chute were increased in the Workshop Treatment, from average 251 Lux in Control Treatment and Mufflers Treatment to 583 Lux in the corner of the single file chute, and from 50 Lux to 1300 Lux at the first vertical door in the single file chute, in the Workshop Treatment.

**c) Electric Prod Use**

As shown in Table 11, over all treatments an average of 83.4% of the cows were subjected to the use of the electric prod at least once, in the stunning-box area just prior to stunning. Almost all of the cull cows were subjected to the electric prod in both Control (98.1%, n=213) and Mufflers Treatments (94.2%, n=190). After the humane handling workshop, the use of the electric prod was reduced significantly (to 66.9%).

**d) Alternative Prod Use (Nested within Workshop Treatment only)**

As shown in Table 11 the hollow cane (alternative prod) was used on an average of 26.3% of the cows in the Workshop Treatment, as compared to no prod of any kind being used on 6.9%, and the electric prod on 66.9% of the cows.

Two of the staff used significantly different (P<0.001, n=320) options in moving cows into the stunning-box after the workshop on humane handling of cattle. One individual opted to use no prod of any kind on 8.1%, the hollow cane on 2 %, and the electric prod on 89.9% of the cows he stunned (n=197). The other individual opted to use no prod of any kind on 4.9%, the hollow cane on 65%, and the electric prod on 30.1% of the cows he stunned (n=123).

**4.5 Tables of Results:**

**Table 1.** Proportions of Cull Holstein Cows Who Were Lactating, Pregnant and Mature in Age at the Time of Slaughter, in Each Treatment Group and All Groups Together.

| Treatment | (n) | % Lactating | (n) | % Pregnant | (n) | % Mature |
|---|---|---|---|---|---|---|
| CONTROL | 210 | 77.6 | 107 | 15.9 | 203 | 70.4 |
| MUFFLERS | 190 | 81.1 | 102 | 13.7 | 188 | 81.4 |
| WORKSHOP | 345 | 86.1 | 67 | 17.9 | 267 | 91 |
| ALL | 745 | **82.4** | 276 | **15.6** | 658 | **81.9** |

**Table 2.** Proportions of Body Condition Scores of Cull Holstein Cows at the Time of Slaughter, in Each Treatment Group and All Groups Together.

| | | BODY CONDITION SCORE | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| Treatment | n | % of cows within treatment | | | | |
| CONTROL | 209 | 28.7 | 44 | 20.1 | 5.7 | 1.4 |
| MUFFLERS | 188 | 54.3 | 29.3 | 8 | 6.9 | 1.6 |
| WORKSHOP | 347 | 42.1 | 26.5 | 17.6 | 10.7 | 3.2 |
| ALL | 744 | 41.4 | 32.1 | 15.9 | 8.3 | 2.3 |

**Table 3.** Proportions of Cull Holstein Cows Originating from Different Parts of the Atlantic Geographic Region, in Each Treatment Group and in All Groups Together, and including the Approximate Average Distance Traveled from Each Part of the Region.

| Treatment | n | MAINE [469 km] | N.S. [210 km] | N.B. [198 km] | P.E.I. [159 km] | Unknown |
|---|---|---|---|---|---|---|
| CONTROL | 213 | 16.4 | 28.2 | 14.1 | 15 | 26.3 |
| MUFFLERS | 190 | 33.2 | 33.2 | 15.3 | 17.4 | 1.1 |
| WORKSHOP | 351 | 24.8 | 33.3 | 12.5 | 18.5 | 10.8 |
| ALL | 754 | 24.5 | 31.8 | 13.7 | 17.2 | 12.7 |

[ ]: average distance to abattoir
Values are percentages of treatment total

**Table 4.** Average Dressed Weights (net pounds) of the Carcasses of Cull Holstein Cows and Average Time Interval Each Cow Spent in the Single File Chute, in Each Treatment Group and All Groups Together.

| VARIABLE | Treatment | n | Mean±SEM | Prob>F |
|---|---|---|---|---|
| Carcass Weight | CONTROL | 213 | 581.7±7.8 | |
| (Net Lbs) | MUFFLERS | 190 | 565.9±8.1 | |
| | WORKSHOP | 291 | 568.0±6.0 | |
| | ALL | 694 | **571.7**±4.1 | ns |
| Time in Chute | CONTROL | 207 | 6.0±0.2 *a* | |
| (Minutes) | MUFFLERS | 111 | 6.1±0.2 *a* | |
| | WORKSHOP | 261 | 4.8±0.1 *b* | |
| | ALL | 579 | **5.5**±0.1 | **0.0001** |

Values are means ±SEM
*a, b* : values ±SEM within the same column not sharing a superscript are significantly different (P<0.0001)

**Table 5.** The Effects of Mufflers Installed on Noisy Air-Relief Valves of Pneumatic Doors in the Single File Chute and A Workshop on Humane Handling of Cattle on Packed Cell Volume (PCV)%, Cortisol, Creatine Phospho-kinase (CPK), pH 45 and pH Ultimate in Cull Holstein Cows at the Time of Slaughter.

| | TREATMENT MEANS ± SEM | | | | |
|---|---|---|---|---|---|
| Response | Control | Mufflers | Workshop | All Treatments | Prob < |
| PCV % | (n=172) | (n=184) | (n=269) | (n=625) | |
| | 36.1± 0.48 *ab* | 36.7± 0.43 *a* | 35.0± 0.34 *b* | 35.8± 0.24 | **0.0063** |
| Cortisol | (n = 70) | (n = 69) | (n = 70) | (n = 209) | |
| ng/ml | 22.9±1.6 | 20.3±1.9 | 23.4±1.9 | 22.2±1.0 | ns |
| CPK | (n = 70) | (n = 69) | (n = 70) | (n = 209) | |
| U/L | 518.8±81.1 *b* | 573.7±59.4 *b* | 960.0±126.3 *a* | 684.7±55.4 | **0.0016** |
| pH 45 min | (n = 153) | (n = 157) | (n = 224) | (n = 534) | |
| | 6.71±0.02 *b* | 6.73±0.02 *b* | 6.82±0.01 *a* | 6.76±0.01 | **0.0001** |
| pH ultimate | (n = 182) | (n = 157) | (n = 237) | (n = 576) | |
| | 6.14±0.02 | 6.18±0.03 | 6.12±0.02 | 6.14±0.01 | ns |

*a*,*b*: values within the same row not sharing a superscript are significantly different (Duncan's multiple range test)

**Table 6.** The Effects of Mufflers Installed on Noisy Air-Relief Valves of Pneumatic Doors in the Single File Chute and A Workshop on Humane Handling of Cattle on the Occurrence of Balking, Struggling, Attempting to Turn Around in the Single File Chute, Trembling and Vocalizing in Cull
Holstein Cows at the Time of Slaughter.

| | CONTROL | | MUFFLERS | | WORKSHOP | | All Trts | | |
|---|---|---|---|---|---|---|---|---|---|
| Behaviour | n | Freq | n | Freq | n | Freq | n | Freq | Chi-sq P< |
| BALKING | 213 | **62.4** | 190 | **62.6** | 349 | **53.6** | 752 | 58.4 | **0.046** |
| STRUGGLING | 213 | 48.4 | 190 | 45.8 | 349 | 53.3 | 752 | 50 | ns |
| TURNING | 213 | **31.5** | 190 | **34.2** | 349 | **25.5** | 752 | 29.4 | **0.078** |
| TREMBLING | 213 | 37.1 | 190 | 35.3 | 349 | 37 | 752 | 36.6 | ns |
| VOCALIZING | 213 | 7.5 | 190 | 10.5 | 349 | 5.7 | 752 | 7.4 | ns |

values indicate percentage of cows within each treatment exhibiting the behaviour

**Table 7.** Effects of Geographic Origin Within the Region Served by an Abattoir, Body Condition, Lactation State, Age and Order of Entry Within Small Groups Herded into the Single File Chute on Packed Cell Volume% and Plasma Cortisol in Cull Holstein Cows at the Time of Slaughter.

| | | Packed Cell Volume % | | | Cortisol ng/ ml | | |
|---|---|---|---|---|---|---|---|
| Variable | | n | Mean±sem | Prob < | n | Mean±sem | Prob |
| ORIGIN | U.S | 171 | 33.9±0.5 | *b* | 67 | 18.5±1.9 | *b* |
| | N.S. | 204 | 36.3±0.4 | *a* | 59 | 23.5±1.9 | *ab* |
| | N.B | 91 | 36.2±0.6 | *a* | 29 | 28.3±3.1 | *a* |
| | P.E.I. | 116 | 36.8±0.5 | *a* | 38 | 20.8±1.7 | *b* |
| | All | 582 | 35.8±0.2 | **0.0001** | 193 | 22.0±1.0 | **0.02** |
| BCS | 1 | 253 | 33.6±0.4 | *c* | 92 | 20.5±1.4 | |
| | 2 | 200 | 36.1±0.4 | *b* | 64 | 24.6±2.3 | |
| | 3 | 96 | 37.8±0.5 | *ab* | 31 | 20.2±2.2 | |
| | 4 | 52 | 39.8±0.6 | *a* | 14 | 27.1±3.6 | |
| | 5 | 15 | 39.9±1.4 | *a* | 5 | 19.1±7.5 | |
| | All | 616 | 35.8±0.2 | **0.0001** | 206 | 22.5±1.0 | ns |
| LACTATION | 0 | 105 | 37.9±0.5 | | 32 | 21.8±1.9 | |
| | 1 | 512 | 35.4±0.3 | | 175 | 22.3±1.2 | |
| | All | 617 | 35.8±0.2 | **0.0001** | 207 | 22.0±1.0 | ns |
| AGE | Young | 108 | 37.3±0.5 | | 32 | 19.9±2.1 | |
| | Mature | 500 | 35.4±0.3 | | 170 | 22.5±1.2 | |
| | All | 608 | 35.8±0.2 | **0.0029** | 202 | 22.1±1.0 | ns |
| ORDER of | 1 | 134 | 36.0±0.5 | | 53 | 17.7±1.4 | *c* |
| ENTRY | 2 | 127 | 36.2±0.5 | | 44 | 22.3±2.5 | *abc* |
| | 3 | 124 | 36.3±0.5 | | 35 | 19.8±1.8 | *bc* |
| | 4 | 103 | 35.8±0.6 | | 32 | 25.1±3.5 | *ab* |
| | 5+ | 137 | 34.9±0.6 | | 45 | 27.3±2.3 | *a* |
| | All | 625 | 35.8±0.2 | ns | 209 | 22.2±1.0 | **0.014** |

BCS:Body condition Score
Values are means±SEM
a,b,c: means±SEM within column not sharing a superscript are significantly different

**Table 8.** Effects of Geographic Origin Within the Region Served by an Abattoir, and Body Condition on pH 45, pH ultimate and Dressed Weight (net pounds) of the Carcasses of Cull Holstein Cows at the Time of Slaughter.

| | | | pH 45 | | | pH ultimate | | | Dressed Weight | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | | **n** | **Mean±sem** | **Prob <** | **n** | **Mean±sem** | **Prob<** | **n** | **Mean±sem** | **Prob <** |
| ORIGIN | U.S | 146 | 6.81±0.02 | *a* | 163 | 6.25±0.02 | *a* | 184 | 529.7±7.5 | *b* |
| | N.S. | 174 | 6.74±0.02 | *b* | 185 | 6.15±0.02 | *b* | 223 | 587.5±6.6 | *a* |
| | N.B | 72 | 6.72±0.02 | *b* | 73 | 6.05±0.03 | *c* | 97 | 591.7±12.1 | *a* |
| | P.E.I. | 90 | 6.80±0.02 | *a* | 95 | 5.99±0.03 | *c* | 120 | 581.8±9.4 | *a* |
| | All | 482 | 6.77±0.01 | **0.0033** | 516 | 6.14±0.01 | **0.0001** | 624 | 571.7±4.1 | **0.0001** |
| BCS | 1 | 206 | 6.82±0.02 | *a* | 221 | 6.27±0.02 | *a* | 282 | 512.0±4.5 | *e* |
| | 2 | 180 | 6.73±0.02 | *ab* | 190 | 6.12±0.02 | *b* | 221 | 567.8±6.0 | *d* |
| | 3 | 83 | 6.71±0.02 | *b* | 92 | 6.02±0.03 | *bc* | 107 | 627.8±8.9 | *c* |
| | 4 | 44 | 6.75±0.04 | *ab* | 47 | 5.97±0.04 | *c* | 57 | 722.0±11.0 | *b* |
| | 5 | 12 | 6.73±0.05 | *ab* | 17 | 6.04±0.08 | *bc* | 17 | 758.3±21.2 | *a* |
| | All | 525 | 6.77±0.01 | **0.0001** | 387 | 6.15±0.01 | **0.0001** | 684 | 571.7±4.1 | **0.0001** |

BCS: Body Condition Score
Values are means±SEM.
a,b,c: means±SEM within column not sharing a superscript are significantly different

**Table 9.** Effects of Lactation State, Age and Order of Entry Within Small Groups Herded into the Single File Chute, on pH 45, pH ultimate and Dressed Weight (net pounds) of the Carcasses of Cull Holstein Cows at the Time of Slaughter.

| | | pH 45 | | | pH ultimate | | | Dressed Weight | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variable | | n | Mean±sem | Prob < | n | Mean±sem | Prob< | n | Mean±sem | Prob < |
| LACT | No | 92 | 6.71±0.02 | | 100 | 6.11±0.03 | | 122 | 615.8±11.6 | |
| | Yes | 436 | 6.77±0.01 | | 469 | 6.15±0.01 | | 563 | 563.1±4.3 | |
| | All | 527 | 6.76±0.01 | **0.0147** | 569 | 6.14±0.01 | **0.0006** | 685 | 571.7±4.1 | **0.0001** |
| AGE | Young | 92 | 6.74±0.02 | | 99 | 6.15±0.03 | | 119 | 517.3±9.2 | |
| | Mature | 426 | 6.77±0.01 | | 461 | 6.15±0.01 | | 536 | 584.1±4.6 | |
| | All | 518 | 6.76±0.01 | ns | 560 | 6.14±0.01 | ns | 655 | 571.7±4.1 | **0.0001** |
| ORDER | 1 | 109 | 6.76±0.02 | | 113 | 6.14±0.03 | *ab* | 146 | 584.5±8.8 | *a* |
| of | 2 | 104 | 6.78±0.02 | | 115 | 6.15±0.03 | *ab* | 137 | 577.2±9.8 | *ab* |
| ENTRY | 3 | 113 | 6.75±0.02 | | 117 | 6.11±0.03 | *b* | 13 | 573.4±9.5 | *ab* |
| in | 4 | 102 | 6.76±0.02 | | 102 | 6.10±0.03 | *b* | 114 | 575.2±10.5 | *ab* |
| Chute | 5+ | 116 | 6.77±0.02 | | 129 | 6.21±0.03 | *a* | 143 | 549.6±8.1 | *b* |
| | All | 544 | 6.76±0.01 | ns | 576 | 6.14±0.01 | **0.0678** | 553 | 571.7±4.1 | **0.074** |

Lact: Lactation state
Values are means±SEM.
a,b,c: means±SEM within column not sharing a superscript are significantly different.

**Table 10.** Average Air Temperatures Outdoors and in the Single File Chute, Relative % Humidity, Noise Levels(dB) inside the Abattoir Barn, Peak Noise Levels in the Stunning-Box Area, Light Levels (Lux) in the Curved Corner and at the First Pneumatic Door of the Single File Chute during the Control Period, After the Mufflers Were Installed on Pneumatic Doors in the Single File Chute and After a Workshop was Conducted in Humane Handling of Cattle.

| Treatment | Temp Out ° *C* | Temp SFC ° *C* | %Hum Outside | %Hum Chute | Noise in Barn | PeakNoise *dB* | LtCnCh *Lux* | LtFs Dr *Lux* |
|---|---|---|---|---|---|---|---|---|
| CONTROL | 20 | 24 | 74% | 76% | 85 dB | 115 dB | 238 | 50 |
| MUFFLERS | 23 | 23 | 68% | 72% | 84 dB | 104 dB | 264 | 49 |
| WORKSHOP | 23 | 24 | 70% | n/a | 78 dB | 98 dB | 583 | 1300 |

Peak noise: at approach to stunning-box.
LtCnCh: light level at corner of chute.
LtFsDr: light at first vertical pneumatic door in chute

**Table 11.** Proportions of Cull Holstein Cows Who Were Not Prodded with Any Herding Device or were Prodded with an Electric Prod or were Prodded with an Alternative Device (Hollow Tassel Cane) in the Stunning-box Area at the Time of Slaughter.

| | | PERCENTAGE | | |
|---|---|---|---|---|
| Treatment | n | No Prod | Electric Prod | Hollow Cane |
| CONTROL | 213 | 1.9 | 98.1 | n/a |
| MUFFLERS | 190 | 5.8 | 94.2 | n/a |
| WORKSHOP | 345 | 6.9 | **66.9 ***** | 26.3 |
| All | 748 | | 83.4 | |

Values are percentages of treatment total.
*** Chi-sq Prob < 0.001

**Table 12.** Significant (P<0.05) Pearson Correlation Values (r) and Probability Values for Packed Cell Volume (PCV)%, pH 45, pH ultimate, Dressed Weight of Carcass, Cortisol, Creatine Phospho-kinase (CPK) and Time Spent in the Single File Chute, of Cull Holstein Cows at the Time of Slaughter

| | | PCV % | pH 45 | pH u | Dressed Wt | Time in Chute |
|---|---|---|---|---|---|---|
| PCV % | n | | | | | |
| | Prob < | | | | | |
| | r | | | | | |
| pH 45 | n | 496 | | | | |
| | Prob < | **0** | | | | |
| | r | **-0.24** | | | | |
| pH ult | n | 531 | 485 | | | |
| | Prob < | **0** | **0** | | | |
| | r | **-0.36** | **+0.17** | | | |
| Dressed Weight | n | 621 | 531 | 573 | | |
| | Prob < | **0** | **0** | **0** | | |
| | r | **+0.32** | **-0.18** | **-0.35** | | |
| Cortisol | n | 202 | 162 | 171 | 209 | 173 |
| | Prob < | ns | ns | ns | ns | **0.0008** |
| | r | | | | | **+ 0.25** |
| CPK | n | 202 | 162 | 171 | 209 | 173 |
| | Prob < | ns | ns | ns | ns | **0.0007** |
| | r | | | | | **-0.26** |
| Time in Chute | n | 529 | 448 | 500 | 575 | |
| | Prob < | ns | ns | **0.0398** | ns | |
| | r | | | **+0.09** | | |

r: Pearson correlation coefficient.
ns: non-significant.

# Chapter 5. Discussion

The following discussion covers the six major themes that arose during the course of my research. Each one combines relevant results gleaned from all ten responses used in the study to indicate fear and stress in cull Holstein cows during the immediate pre-slaughter period.

## 5.1 Why Cull Dairy Cows are in Poor Condition at Time of Slaughter

Characteristic features of cull Holstein cows at the abattoir appear consistent with what is known about the composition of the cull dairy cow population that leaves the farm. As shown in Table 1, most of the cows were **mature** in age (81.9%), an observation that was also made in Milian-Suazo et al. (1988). There are many reasons why so many of the cull Holstein cows in the study were thin, lactating, and not pregnant.

The stress of early **lactation** and accompanying state of negative energy balance during this stage may partially account for the generally poor condition of the cows. As pointed out earlier, Bouchard et al. (1997) found that the average interval from calving to culling for cows with reproductive problems, was 174 days. At this point in the lactation curve most Holstein cows (especially **high producers**) could be expected to be showing dairyness (lean and angular) and not regaining much weight lost from early lactation stress. Many of the cows observed who were in BCS 1 and 2, therefore, may have been non-pregnant mid-lactation high-producers whose mature age or feet and leg or udder problems may have led to the final decision to cull them from the herd. Other cows in poor body condition may have recently been suffering from **disease** such as milk fever, dystocia, retained placenta, metritis, ketosis, and mastitis. The cows in better body condition may have been the ones culled for low milk production, or mastitis or udder problems (and some in this group may have been highly-valued cows who were dried off in order to improve the chances of over-coming breeding problems but who could not be settled in calf after all, ...the speculation list is long).

The average dressed weight of the cows provides some corroboration with the observation of low BCSs. Cull Holstein cows dressed out at only 77% of the normal carcass weight of a beef steer or heifer. An average dressed weight of 571.7 lbs (260 kg) is only 43% of average live weight (600 kg/1320 lbs) of a Holstein cow. Fed beef cattle, by comparison, dress out at an average rate of 53-55% (McCaughey and Cliplef, 1996) typically in Canada, and at 56-58% (740 lb. carcass from a 1300 lb live weight) at this particular abattoir (Ratcliffe, 1997). Another reason for the low dressing percentage may be **bruising** (therefore trimming), and "**shrinkage**" resulting from transportation stress. The study was able to confirm that cull dairy cows originating from the entire Atlantic region, including the State of Maine, are transported to this one large abattoir. The plant therefore services a **large geographic region**, which indicates that these animals, most of whom are lactating when they leave the farm, are also subjected to the stress of long-distance transportation.

Dark-cutting carcasses result from **low glycogen** content in muscles post mortem, and 65% of the cows in this study had pHu values > 6.0, therefore in the dark-cutting category. Below, I will discuss the relationship found in the study between body condition and pHu. However, there are possibly other reasons besides poor body condition that cows in general might produce DFD carcasses. Cows, by reason of their sex, may tend toward producing DFD carcasses. Scanga et al. (1998) found that DFD was affected by differing facility construction and cattle-handling procedures, adverse weather conditions (too cold or too hot), and that heifers were more prone to producing DFD than steers, due possibly to **estrogen** secretion. On the other hand, Kenny and Tarrant (1988) found that muscle glycogen loss and pHu was associated with the number of times a heifer mounted another. This explanation is more consistent with conventional wisdom, as the link between agonistic behaviour, physical exertion and DFD is well-documented. (Informally, I observed virtually no mounting behaviour in the single file chute, nor did I observe much mounting in the lairage pens in the morning prior to the day's kill operation.) One viable explanation for high pHu in these cows may be the relationship between **bruising** and pH in the muscle *post mortem*. Cull cows may be handled more roughly in the pre-slaughter period than fed beef cattle due to their slower movement, and bruising may therefore be increased. Jarvis et al. (1995) found that bruising of carcasses was higher in cattle who came through cattle markets rather than directly from farms, and that the use of driving instruments caused bruising. When an animal is bruised, the bruised site accumulates exudate antemortem, and could cause an elevation of pH values or maintenance of high pH *post mortem* (Gregory 1996).

## 5.2 Evidence of Cull Dairy Cows' Vulnerability

Results from this study show concrete evidence that cull Holstein cows constitute a vulnerable category of livestock at an abattoir, indeed throughout the entire period of the culling process from the time the cow leaves the farm until she is slaughtered.

Considerable escape/avoidance behaviour was exhibited in the forms of balking, attempting to turn around in the single file chute, and vocalizing in the stunning-box area immediately prior to stunning (Table 6). A combination of physical stress and overt fear behaviour was shown in the forms of struggling and trembling just prior to slaughter. For example, the occurrence of trembling in just over one-third of the cows (36.6%), may indicate a high level of epinephrine release.

At an average of 35.8%±5.9 , PCV % was found to be higher than the normal average value of 33.6±5.2% for adult cattle (Jain, 1986). It was also higher than the average PCV% for lactating Holstein cows at the Nova Scotia Agricultural College, which was 29.6% (n=40). There are several possible explanations for this.

A number of studies (Jarvis et al., 1996; Tarrant et al., 1992) indicated that fed beef cattle who were transported long distances and deprived of water tended to be **dehydrated** at the time of slaughter. It was, therefore, anticipated at the outset of this study that the cows may have shown a tendency to be dehydrated, as they had been transported from all over the Atlantic region, during the hot summer months, and in a stage of lactation. This may indeed have been the case, since the values found in this study were higher than those of both the textbook reference and the local baseline. However, since the difference is not large, it may be that, during the lairage period, most of the cows were able to gain access to sufficient water to re-hydrate themselves if they were indeed dehydrated at the time of arrival at the abattoir. In this study the cows originating from Maine (the farthest distance away from the abattoir, Table 3) had the lowest PCV%. Since this is in contrast to other studies (e.g., Jarvis et al., 1996) which have found that there was a trend for PCV% to increase with distance traveled from markets, I suggest that perhaps PCV% in cull dairy cows is affected by additional factors such as lactation and body condition, and age.

Milk production is known to place metabolic stress on a cow, so it was not surprising that the lactating cows had a lower PCV% than non-lactating cows (Table 7). Cows who are in poor body condition would be expected to have lower PCV% than cows with more condition, because a healthy body has a better ability to produce and replenish blood cells than one that is thin or emaciated. As shown in Table 7, the emaciated cows (BCS=1) in this study indeed had the lowest PCV%, at 33.6±0.4%, which was significantly lower than all the other cows in BCS>1. Older cows also would be expected to be less healthy in general than younger cows, and consequently have lower PCV%, and indeed it was the case in this study that the mature cows had an average of 35.4±0.3% as compared to young cows, who had an average of 37.3 ±0.5%.

Cows who were mature, lactating, and in poor body condition, also may have been tending toward **anemia**. Some cows appeared lethargic and weak, which are symptoms of anemia (Merck, 1991). Anemia refers to a decreased concentration of functional red blood cells. It results from a loss of red blood cells, or from deficiencies in folic acid or vitamin B12, or inadequate hemoglobin synthesis, or premature breakdown of red cells (Frandson, 1986).

Another possible explanation for higher-than-normal PCV % values observed may reflect an elevation at the time of stunning as compared to that in lairage, due to **splenic contractions related to fear** (Jain, 1986). Since the present study was conducted using a policy of non-invasive observation of cows, blood was not collected at various points in time following the cows' arrival at the abattoir, so an exploration of this potentiality was not possible.

Creatine phospho-kinase was found, on average at 684.7±55.4U/L, to be at a borderline pathological level (according to the Atlantic Veterinary Diagnostic Lab guideline) for cattle. A baseline normal value (on-farm, pre-transport) for fed beef steers, used by Tarrant et al. (1992) was 34 U/L (n=144). The Holstein cows in the NSAC dairy herd showed a mean CPK value of 193.2 U/L.

Perhaps the cows who were quiescent in their behaviour, possibly because of exhaustion and illness, were cows who were **more easily pushed around** by stronger cows and by human handlers, and therefore could be expected to be bruised by events that occurred prior to entering the chute, such as transportation injuries.

Another speculation regarding the higher values of CPK in the Workshop Treatment is that those cows may have been subjected to a higher **stocking density** than the cows in the other two treatment groups. Tarrant et al. (1992) found that plasma cortisol, glucose and creatine kinase were elevated increasingly with stocking density in steers after transport. Stocking density was not investigated in my study, but in future research this factor should be studied.

**Starvation** and/or **exhaustion** can contribute to CPK release. Therefore, perhaps the results I obtained with cull dairy cows are an indication that the cows were either suffering from **physical injury** due to cumulative physical stress during the entire process of handling after leaving the farm up until slaughter, or were utilizing their own flesh to survive, considering their poor body condition, and the likely deprivation of forage feed since leaving the farm. Higher than normal values for CPK may have been due to **bruising damage** to muscles causing the release of enzymes into the bloodstream, and cull cows are known to have more bruising than fed beef cattle. One viable explanation for high pHu in these cows may be the relationship between bruising and pH in the muscle *post mortem*. (Jarvis et al., 1995; Gregory, 1996).

**5. 3 The Relationship Between Body Condition and DFD Carcasses**

The average pHu value was in the Dark Firm Dry (**DFD**) category, at pH 6.14±0.01 (Table 5). Dark-cutting carcasses are an indication that the cattle were stressed physically and psychologically, and/or in very poor condition. A high pHu in the carcasses of these cows is consistent with conditions found in the study, including the cows' display of considerable balking, struggling, turning, trembling and vocalizing in the stunning-box area prior to slaughter; and the emaciated state of many cows.

Dark-cutting carcasses of fed beef cattle occur in a small percentage of the fed beef cattle population (1-3%), and are generally caused by agonistic behaviour, involving physical exertion, especially mounting of other cattle, in the pre-slaughter period. Agonistic behaviour was not recorded in the present study. During the preparatory period prior to the commencement of the study, very little of this type of behaviour was observed in the cull cows while they were in the single file chute. However, physical exertion is involved in three of the behavioural responses chosen for the present study (balking, struggling, and attempting to turn around in the single file chute). But overt physical exertion observed in the immediate pre-stunning period was not shown to affect pHu. Meanwhile, cows in the poorest body condition tended to have the highest pHu (Table 8), and these cows may have been too weak to use an active strategy such as turning in the chute. A complete ethogram of the behaviour of this category of cattle would possibly shed more light on key mechanisms which contribute to more or less stress in these animals in the context of a pre-slaughter environment. In a future study, perhaps it would be helpful to observe cull dairy cows from the time they are unloaded at the abattoir, during the lairage period overnight, and throughout the entire period they are in the single file chute up to the point of stunning, so as to record any agonistic behaviour. Kondo and Hurnik (1990) found that the greatest number of agonistic encounters happened immediately following re-grouping of lactating dairy cows (in a farm situation), and that they diminished from then on. This may explain why I observed (informally) very little if any agonistic behaviour among the cows in the lairage pens just prior to the herding activity of the morning kill.

It is known that pHu is a direct reflection of the amount of **glycogen** present in the muscle ante-mortem, and the extent of glycolysis post-mortem, in which more or less lactic acid is produced, to lower the pHu only as far as the amount of glycogen present permits. The cull cows may have been severely **glycogen-deficient** because of their poor body condition as well as stress from other sources such as transportation and disease. Depleted glycogen reserves in these animals may explain the fact that there was no treatment effect, because there may not have been enough glycogen present for fear to manifest itself in the muscle no matter how little or how great the fear. Epinephrine release may have little effect on pHu, where there simply isn't enough glycogen to act on to make any difference one way or the other. It would be useful to know what the pHu value is in the carcasses of emaciated cows who are in a familiar home-farm environment at the time of stunning. Future research will perhaps shed light on the relationship between body condition and glycogen levels in various contexts.

### 5. 4 Reducing One Sudden Loud Noise

There were no significant differences found between the Control and the Mufflers Treatment with regard to the five behaviours, nor the five physiological indicators measured in this study. The mufflers effectively reduced the noise level of the air-exhaust valves to the extent that the former loud, hissing noise that was produced was virtually eliminated (115 dB to approximately 60 dB). Other noises present included that from operating equipment such as the overhead shackle-chain return track, serum separators running at high speed in the near-by blood-room, a loud-speaker which intermittently erupted with announcements, a large auger turning in a long trough in the kill-floor area (to carry offal to a holding tank), and a radio playing (up to115 dBs at times) close to the entrance of the stunning-box. These and other noises were emitted into a large, open space with a high steel ceiling and no insulation in the area of the stunning-box to absorb some of the sound, and consequently the environment was still very noisy following the installation of the mufflers on the air-exhaust valves.

Reasons why only a single source of noise was reduced, rather than several in the stunning-box area included financial constraints, the practical limitations of a complex commercial abattoir, and to address the question of whether removing one major irritant to the cows would relieve their stress to some extent. The fact that it did not appear to do so in this study, suggests that it may be necessary to reduce total noise in the vicinity of the cows, before any changes in the same fear responses may be observed.

Although no benefit was evident as far as the cows were concerned ($P>0.10$), the employees expressed their preference for the mufflers over the former arrangement. Therefore, it was at least a positive benefit to the workforce who were previously exposed to the loud hissing noise of the pneumatic system. This benefit may, in the long run, translate to improved handling of the cattle.

### 5.5 Importance of Humane Handling Practices at an Abattoir

The Workshop Treatment resulted in a significant reduction of two indicators of fear behaviour (balking and turning), a reduction in the average time the cow spent in the single file chute prior to stunning, and improvements in lighting, combined with the removal of the sudden loud noise of the air exhaust valves due to the installation of mufflers in the previous treatment. Although modest, the reductions in balking and turning are indications that the fear the cows were experiencing just prior to stunning may have been reduced, and that this reduction resulted after only one workshop session with staff. The implication, therefore, is that a further reduction in the cows' fear may be attained through a combination of more up-grading of training for personnel, and physical improvements to the abattoir such as installing non-slip flooring in the stunning-box and eliminating air blowing in the cows' faces at the entrance to the stunning-box.

**Electric prodding** was significantly reduced after the workshop (66.9% from an average of 96.3% in the two prior treatments)(Table 11). With less use of the electric prod, there was less balking. It is possible that it was balking that had an effect on electric prodding. The sequence of these two events were not recorded in the study, and therefore it is not possible to resolve this question without further data collection. However, it may still be plausible to conclude that it was electric prodding that affected frequency of balking because reduction of electric prod use was a primary focus of the workshop, and the results indicate that it was successful to a limited degree in reducing both electric prod use and frequency of balking. Although the results found in this study suggest that one workshop did appear to have a small impact, it is clear that further education and an improvement in the standard of excellence are required for any further improvements to be made in electric prod use reduction. The observation of prod use was only noted for one area of the chute (the entrance to the stunning-box), therefore, it is not known what the total frequency of prodding per cow was for the entire interval of time the cow spent in the single file chute. Electric prodding of cows was observed informally, however, to occur at several other points along the route from the holding pen and throughout the chute. Cull dairy cows were observed (informally) to move more slowly than fed beef cattle as they were moved from the lairage pens into the chute. This seemed to annoy human handlers at the plant and there appeared to be a perception by them that cull cows slow down "normal" line-speed through the single file chute and stunning-box. It is interesting to note that although a commonly-heard remark by personnel was that the dairy cows were "slow, stupid and lazy", the mean time they spent in the single file chute in the Workshop Treatment was actually slightly less (4.8 min vs 5.1 min) than the mean time spent by beef steers and heifers (n=95) during the same treatment period.

The reduction in electric prod use which occurred in the Workshop Treatment had no effect on cortisol levels. This may have occurred due to insufficient time for activation of cortisol relative to electric prodding in the chute. Also, this is consistent with what is known about cortisol and electric shock in cows. Lefcourt et al. (1986) found that glucocorticoids in dairy cows were not affected by increases in shock intensity, which is unlike other species such as rats and rhesus monkeys.

The **individual person** handling and stunning the cows appeared to have had a significant effect on frequency of balking, which may be an indication that human attitudes and on-the-job performance play an important role in affecting cow behaviour in this context. This underlines the importance of having trained personnel who can demonstrate a willingness to put their training into practice and succeed at it.

Handling practices include physical lay-out and equipment as well as direct handling of animals. Integrated management of all these factors is important. For instance, **physical design** factors as well as direct handling of the cows within the stunning-box may have influenced the stability of the cows (and therefore the frequency of struggling) at the point just prior to stunning. The floor of the stunning-box was concrete, and had grooves cut into it which had become worn and smooth over time, and it was sloped sideways slightly toward the kill-floor. This contributed to a **slippery surface** for the cows to stand on. One consequence of this may have been in evidence according to the occurrence of struggling by cows in the stunning-box area. Struggling occurred at a frequency of 50% on average, indicating a considerable loss of the physical stability of the cow, which in turn may have contributed to a fear of losing her footing and falling.

Another factor I observed that may have created problems for the cow's physical stability in the stunning-box, was the practice of **acquiring the ear-tag number** of each individual cow as she came into the stunning-box. The person working as the stunner of the cows typically had to touch the cow, sometimes holding the ear, in order to read the number on the ear-tag. It may be desirable from both welfare and efficiency perspectives to acquire the ear-tag numbers after the cow is dead on the rail. It is interesting to note that I observed that if the cow was very agitated during the time the stunner was attempting to get the ear-tag number (struggling excessively and attempting to turn around often), the stunner would often place his hand on the cow's back, close to her shoulders, resulting in the cow ceasing or reducing the previous agitated behaviour. This may be yet another new challenge to her coping mechanisms, and the cow may be attempting to re-orient herself in response to that challenge.

Improvements made in the single file chute area, such as **brighter lighting** and r**emoval of hissing noise** of pneumatic doors, in combination with **improved handling** of the cows through increased awareness of flight zone and point of balance in moving cattle forward, may account for the reduction in turning. It should be noted that the reduction in turning frequency may also have led to the significantly reduced time spent in the single file chute which occurred in the Workshop Treatment as compared to the two earlier treatments

(Table 4). This was a 20% reduction in time for this management procedure, which provided a benefit to the cows by reducing fear behaviour, as well as potential plant benefits of improving the line-speed in the chute.

The decrease in PCV% in the Workshop Treatment may be due to reduced fear in the cows, as a result of the possibility that fewer cows produced splenic contractions as compared with the two earlier treatments.

The increase in pH 45 found in the Workshop Treatment was quite small in practical terms (Table 5), but may provide some insight into the effects of reducing pre-slaughter fear. For instance, the difference may be explained by a **slight reduction in Pale Soft Exudative (PSE) conditions**, due to reduced signs of fear in the cows. Acute stress can cause a rapid rate of glycolysis in the muscle, and if this occurs, a greater amount of lactic acid would be expected to build up in the muscle within the first hour post mortem, and consequently the pH in the muscle would be lower than if less rapid glycolysis was occurring. Frequency of balking and turning were significantly reduced in the Workshop Treatment, and this reduction in fear may have led to a pH change in the muscle post mortem, that was slower than what would occur if there was more stress on the cows. Epinephrine acts to speed up glycolysis, and cortisol acts to slow it down. Cortisol levels did not change by treatment in this study, but epinephrine may have been reduced in the Workshop Treatment as evidenced from the reduction of frequency of balking and turning. Slower rate of glycolysis post mortem means higher pH at 45 min in the LD muscle, as compared to a faster rate of glycolysis due to increased fear and the consequent increased epinephrine levels. So it is very tentatively suggested that the higher pH 45 min values found in the Workshop Treatment are consistent with reduction in fear, as evidenced by reduced fear behaviour.

### 5. 6 Indicators of Fear and Assessment of Welfare in Cull Dairy Cows

Several insights were obtained regarding the sensitivity and/or utility of specific indicators of fear for use in the context of pre-slaughter handling of cull cows in an abattoir. A few of those insights are presented here.

It is likely that the reason that some cows were vocalizing in the stunning-box area was due to an alarm, or perceived emergency state. This study has quantified the relationship between a stressor (electric prodding) clearly identified in previous studies with at least one behaviour (vocalization) that may indicate fear. Over all treatments, it was shown that 8.2% of cows vocalized when electrically prodded, and 3.9% vocalized when they were not electrically prodded (P=0.086, n=752). Grandin (1998) suggested that vocalization scoring is a useful practical indicator of welfare in assessing pre-slaughter stress. The scoring system in the USDA survey she conducted in 1996 (Grandin, 1997a), used an occurrence of vocalization (in all cattle types) of <0.5% in the stunning-box area, as an "excellent" score, and <3% as an "acceptable" score for a slaughter plant. In recent audits of slaughter plants in the U.S. and Canada that handle cows, Grandin (1999, unpublished) found that low occurrence of vocalization (in < 3% of cows) corresponded with use of well-trained personnel and minimal distractions in the chute, which minimized the occurrence of balking and the use of the electric prod. The opposite was true in slaughter plants where the occurrence of vocalization was high (>3%). The quality of cattle-handling at the abattoir where the present study was conducted would likely receive a failing grade according to these standards.

I suggest that assessing fear in an individual cow (as opposed to assessing a slaughter-plant's quality of handling cattle overall) poses additional complications. For instance, in my study more cows from Prince Edward Island (P.E.I.) were found to vocalize than cows from other parts of the region (13.9% of cows from P.E.I. vs avg 6.5% from other parts of the region, P=0.026, n=656). This may be have been due to **genetic differences** within the Holstein breed within the region. Perhaps P.E.I. cows are more excitable leading to more agitated behaviour. **Distance** traveled from the farm to the abattoir may have played a role. The estimated distance traveled from P.E.I. was the shortest of all from within the region. P.E.I. cows traveled approximately159 km to the abattoir, vs 198 km from N.B., 210 km from N.S., and 469 km from Maine. These P.E.I. cows perhaps had more energy to attempt to avoid or escape the stressful situation facing them, relative to cows who were transported longer distances and may also have been emaciated, fatigued and suffering from disease such as mastitis.

Although there was a significant reduction in PCV% in the Workshop Treatment as compared to the two earlier treatments, it was not a large difference in practical terms. Also, PCV% was shown to be easily confounded by several intrinsic factors, which are variable in this group of cows. The origin of the cow, the BCS, the lactation state , the age, and the dressed weight of the cow, all significantly affected this response.

The local baseline level of cortisol, determined for lactating Holstein cows from the milking herd at the NSAC was 4.1 ng/ml (n=10). These cows were healthy, and comfortable in familiar surroundings alongside herdmates. This value is consistent with the Schalm's

(Jain, 1986) value of 4.7 ng/ml for lactating cows, and with other research involving dairy cows.

The average cortisol level of abattoir cull Holsteins was found to be 22.2±1.0 ng/ml, which is greater than five times the local baseline level used for the study. As compared to other studies conducted with cattle at abattoirs, however, this level is lower than most, which could be explained by any of the following factors or a combination of them. Most of the cows (82.4%), were **lactating** at the time of death. Previous studies have shown a link between lactation state and cortisol secretion. Cook (1997) found that lactating sheep showed a lower concentration of cortisol in response to acute psychological stress than non-lactating sheep. In Holstein cows, Sartin and Kemppainen (1988) found that high producers secreted a lower concentration of cortisol at d 30 than at d 90 postpartum, and at d 30 compared to non-lactating cows. This may explain why the cows in my study had lower mean values than cattle in previous studies, who were beef cattle and presumably non-lactating for the most part. However, my study found no significant difference in cortisol levels between lactating and non-lactating cows. Perhaps the cows who were thought to be non-lactating were actually lactating to a small degree, or there was too small a number of non-lactating cows in the study.

An **interaction of genetics and previous experience**, combined with the fact that most of the cows were lactating, may explain this lower cortisol value. Holsteins are genetically calm animals (Grandin 1997b), and the relative tameness, and previous exposure of dairy cows to loud noises and human handling as compared to fed beef cattle may account for the difference. However, as previous studies have shown, tameness in animals doesn't necessarily generalize to all procedures and situations, especially if they are highly aversive.

Another possibility is that many of the cows were **seriously depleted** of their energy reserves and ability to cope actively. The cows' mature age and poor body condition, as well as the possibility of pain and injury due to systemic illness such as mastitis or ketosis, or bruising and tissue damage from long transportation and rough handling, may have reduced the cow's ability to respond to the stress. Exhausted cows may be conserving precious energy resources to survive as long as possible. Self-preservation strategies may be different for mature, weak, infirm cattle than for healthy ones, in relation to cortisol release for one thing, as well as behaviour in the face of an emergency situation (passive strategies vs active strategies). A quiet, slow-moving cow may not be less frightened than a cow who is able to attempt to escape from the chute, or vocalize her fear, or she may be more frightened, or equally as frightened. It appears, therefore, that cortisol may not be a useful measure of acute fear in this group of cattle. Also, the cows' adrenal glands may have become depleted as a result of fatigue and prolonged cortisol secretion from the entire culling process. Prolonged stress-induced hypersecretion of cortisol suppresses neural activity because of depletion of energy reserves, and can lead to depression (Brown, 1994).

Cortisol levels seem to have been influenced by **origin** of the cow. One explanation may be that cows subjected to long transportation, especially if they are in poor condition. (cows from Maine had carcasses with significantly smaller dressed weights than from any other parts of the region, Table 8), are simply too exhausted to attempt to cope with the situation, and give up.

Cortisol measurements are known to be **time-dependent** (Lay et al., 1992; Alam and

Dobson, 1986). The fact that I found no significant difference in cortisol levels among treatments, may be explained by the possibility that there was insufficient time while the cow was in the chute, for cortisol to peak. It was anticipated at the outset of the study, that cortisol might already be in circulation when the cows were herded into the single file chute from the larger lairage pens in the barn (due to the increased activity level in the barn during the entire shift compared to the overnight period when the cows were left alone in the large pens) and that therefore the time spent in the chute would be a sufficient period of time for cortisol to peak, prior to stunning. However, it may be that there was too brief a time for cortisol to peak in every cow, due to cortisol not being in circulation at the time the cows were moved into the SFC (first position cows spent an average of 3.9 min in the SFC, while last position cows spent an average of 7.4 min in the SFC). Over all treatments, it was found that **order of entry** into the single file chute significantly affected the level of cortisol (Table 7). Also, a highly significant but very weak positive correlation was found between cortisol and time spent in the SFC (Table 12). This is consistent with other studies which have reported that elevated cortisol levels result from increasing time spent in the single file chute, or race as it is sometimes called (Cockram and Corley 1991). The average time the cow spent in the SFC was affected by treatment (Table 4). Although it was reduced in the Workshop Treatment by 20%, this reduction did not make a difference in terms of cortisol levels across treatments. Therefore, the position of the cow may have affected cortisol level more than the factor of time spent in the chute. One possible explanation may be that the cows in the rear of the small group may have received more handling attention, such as more electric prodding. The more likely explanation is that there was insufficient time for cortisol to peak in all the cows, and that it is therefore an inappropriate indicator of stress in this context.

Another possible explanation for the relatively low cortisol levels I found in this study is that it doesn't matter how high or low the values are because **cortisol may not be a reliable indicator** of stress. Rushen (1991) has argued that conflicting results found in the literature, combined with the issues of whether the metabolic consequence of cortisol are related to unbound rather than total corticosteroids, and the high-frequency low-amplitude pulsatile release of cortisol making it difficult to acquire a representative reading, all contribute to creating doubt about the reliability of cortisol measurements in predicting stress responses.

A final possible suggested explanation of the lower cortisol levels found is the **long time interval between collection of blood and freezing** the plasma. Because the abattoir was a 3-hour drive from the laboratory, and the kill-operation each day took several hours, it was as long as 7 hours between collection and freezing. Although the blood samples were kept on ice the entire time, the length of time before freezing may have caused a decrease in the amount of cortisol still active in the samples.

Enzymes are released quickly into the bloodstream (McGilvery 1979), so it was thought that CPK levels might reflect a reliable response to the acute stress conditions in the pre-stunning period. It was not possible in this study to differentiate between the effects of injury and illness, and acute psychological stress on CPK secretion. The finding that mean CPK levels were significantly different in the Workshop Treatment as compared to the two earlier treatments, but that it was almost double the amount, is perplexing.

A possible reason why CPK was higher in the Workshop Treatment is that there may have been a difference in **technique employed in sticking** the cows according to the individual person doing the job. I did not record this information so I could not test its effect. Because exsanguination is itself a substantial injury, CPK may be acutely sensitive to varying technique.

A mean pH 45 of 6.76±0.01 is a deviation from a normal value (approximately 6.4) in a fed beef carcass. This higher value is consistent with what would be expected in the case of a carcass that will ultimately have a high pHu (**DFD**) condition. Most of the cows were in poor body condition. Although no published information linking body condition and glycogen levels in muscle was found, glycogen levels may have been already quite low at the time they were herded into the chute. Exactly how low they were is not known, because glycogen itself was not measured in this study. Further research regarding this question is suggested. If cows in good body condition have greater glycogen stores in their muscles than cows in poorer body condition it could be expected that glycolysis occurring in the muscle in the first hour post mortem would produce more lactic acid as compared with an animal whose glycogen stores might be more depleted, and would thus produce a lower pH in the muscle at that time. If the cow in good condition was very stressed at the time of slaughter, glycolysis would be speeded up due to epinephrine secretion, and the pH at 45 min might be even lower. Over all treatments, emaciated cows had a significantly higher pH 45 than cows with BCS=3 (Table 8).

Another explanation for the relatively high pH 45 in the Workshop Treatment may be that the thinner carcasses produced higher pH45 because of lower **body temperature**. The rate of pH decline is closely related to muscle temperature shortly after slaughter, with high muscle temperature increasing the rate of pH drop (Pearson in Price and Schweigert 1987). However, I found no significant correlation between muscle temperature and pH 45.

Ultimate pH value in the carcass is a reliable indicator of the cumulative stress, both physical and psychological, that the animal has experienced during the pre-slaughter period. Certain intrinsic factors, by contrast with treatment effect, were shown to significantly affect pHu. In every treatment, both **origin** of the cow and **body condition** significantly affected pHu, and in the Workshop Treatment, **order of entry** into the single file chute was shown to have an influence on it (highest values in last position in chute, Table 9).

Cows whose carcasses had the highest pHu originated in Maine, which is the farthest distance away from the abattoir as compared to cows from other parts of the region. Without knowing the exact origin of the cows within each part of the region, and therefore not knowing the exact distance each cow was transported to the abattoir, it is not possible to make exact comparisons of pHu according to distance from the abattoir. However, in general terms, it appears that **distance** may be a factor, because it was found that BCS was significantly related to origin of the cow. Cows from Maine were in the poorest condition overall, and had the lowest average dressed weights. More cows from P.E.I. had the lowest pHu of all the cows by origin (Table 8), in fact they were the only group of cows whose carcasses, on average, were not dark-cutters. The origin itself may represent certain other factors that were unknown to the present study, such as the possibility that Holstein cows coming from Maine had been treated with **recombinant Bovine Somatotrophin (rBST)** for some period of time prior to being culled out of the dairy herds. rBST is known to have deleterious effects on body condition, among other things (Monsanto 1990).

**Genetic differences** among dairy cows by origin in the region also may account for a difference in pHu. More excitable animals may experience a faster rate of glycolysis, thus reducing glycogen stores resulting in a high pHu in the carcass.

No relationship between pHu and cortisol was found in my study, which supports previous work which has found that indications of stress in meat quality and those in plasma constituents do not necessarily relate to each other. For instance, Tume and Shaw (1992a) found no dark-cutters in a group of cattle which had an average of 44.6ng/ml cortisol at exsanguination, and Warris s(1990) found no difference in cortisol between dark-cutting and normal bull carcasses. Tume and Shaw (1992b) speculated that this is the case because of the transient nature of changes in concentration of blood constituents. I suggest that possibly the time-dependent nature of cortisol response (as was discussed earlier) may not have been adequately taken into account in the studies.

# Chapter 6. Conclusions

## 6.1 Summary of Findings

**a)** Demographic information about the sample of cull Holstein cows: 81.9 % mature in age, 82.4 % lactating, 15.6 % pregnant, 73.5 % in BCS 1 & 2, originating from points within a wide geographic region.

**b)** Cull cows showed evidence of fearfulness at the point of stunning.

**c)** Reducing a single loud noise (air-relief valves on pneumatic doors) decreased dB level but did not affect behavioural or physiological indicators of fear used in the study.

**d)** One workshop on humane handling of cattle had some success in reducing two measures of fear (balking and attempting to turn around in the chute).

**e)** Packed cell volume % was slightly decreased and pH 45 was slightly increased after the workshop on humane handling, possibly due to reduced splenic contractions and reduced pale soft exudative (PSE) conditions, respectively.

**f)** Electric prod use was reduced and the time each cow spent in the single file chute was decreased by 20% after the workshop.

**g)** The number of cows vocalizing was reduced by half when the electric prod was not used.

**h)** There was no treatment effect on concentration of plasma cortisol.

**i)** Most cows' carcasses were classified in the dark firm dry (DFD) category, which indicates depleted energy stores, thus poor welfare.

**6.2 Recommendations for Future Research**:

Further research is required to understand more about the experience of cull dairy cows during the entire culling process. Only when more is known about their behaviour during each stage of this period (such as on the farm prior to transport to market, handling at an auction market, during transportation, and upon arrival and during lairage at an abattoir) will it be possible to make appropriate recommendations for reforms which accurately reflect the needs of cull cows.

Epidemiological studies of cull dairy cows as they present at abattoirs will be required to know more about their health status at the time of death. This work will help distinguish between the reasons that dairy cows are culled in the first place, and the characteristic condition and health of the cows at the time of slaughter. Combined with the ethological work mentioned above, it can describe specific problems, identify relevant food safety issues, and help establish improved protocols for handling this vulnerable type of cattle from farm to stunning-box in an abattoir.

Comparisons of behaviour and carcass characteristics between cull Holstein cows and other types of cattle, such as cull cows of other dairy breeds, cull beef cows, beef steers and heifers handled in the same abattoir conditions would determine both commonalities and differences among these groups in relation to the pre-slaughter experience.

### 6.3 Implications of the Research

My research set out to describe characteristic features and behaviour of cull Holstein cows as they faced their demise at an abattoir. Experimental objectives included testing the effects of one specific stressor, and of training personnel in humane handling techniques aimed at minimizing fear. Removing the loud noise of air-exhaust valves on vertical doors located in the single file chute did not make a detectable difference in the cows' behaviour. However, one workshop with abattoir personnel on humane handling of cattle succeeded with modest reductions in overt fear behaviour.

The study revealed much about a population of cattle that is especially vulnerable to poor welfare during the immediate pre-slaughter period. Cull Holstein cows exhibited poor health that was shown to be consistent with documented reasons for culling cows from dairy herds. The specific ways in which their poor health exacerbated the stressful experience of routine handling practices in an abattoir were quantified in terms of the cows' behaviour and carcass characteristics.

Most cull Holsteins were mature cows in poor body condition, lactating, and not pregnant. Evidence was found to indicate that they were transported from points within a large geographic region, and were likely bruised, fatigued, depleted of energy stores, and fearful as they approached the stunning-box just prior to stunning.

Most carcasses were found to be in the dark-cutting category, indicating depletion of glycogen reserves due possibly to a combination of poor condition and fear. Body condition score was shown to be negatively correlated with ultimate pH of the *longissimus dorsi* muscle. The discovery in this study that cull dairy cows may tend to produce dark-cutting carcasses at a very high rate, is a warning sign that perhaps this group of animals deserves more attention, both from a welfare and a food safety perspective.

Following the workshop in humane handling of cattle, the abattoir began a process to improve handling methods and facilities in the single file chute area. As a result, modest yet positive improvements were made in terms of reducing overt fear behaviour in the cows, reducing electric prod use, and reducing the time required to handle each small group of cows in the chute. The importance of training personnel in handling techniques that reduce cows' fear, hand-in-hand with making strategic physical changes in the plant such as improved lighting, are thus illustrative of key steps in safe-guarding animal welfare right up to the moment of death.

Dairy cows constitute a significant portion of the cull cow population, and this study has contributed some additional knowledge of its physical and demographic make-up. There is sufficient indication from this study that cull dairy cows are suffering beyond what is reasonable given the stressful conditions. Our society and dairy industry have clearly stated that avoidable stress and avoidable pain must not be permitted to occur in food animals. This study has quantified suffering in accordance with the stated criteria of the Voluntary Code of Practice for the Handling and Care of Dairy Cattle and public legislation at the provincial and federal level. It has revealed some disturbing realities about this category of food animal in the context of the pre-slaughter period, and it is hoped that the information it contains will assist in improving conditions for dairy cows during the culling period in general and the pre-slaughter period in particular. Appropriate remedies are required in order to prevent, or at least minimize, the suffering that cull dairy cows face upon being 'disposed of'. Rather than suspend the welfare of these animals during the culling process ‘because they are just going to be killed anyway’, this is precisely the time which warrants our best efforts to safeguard it. Cull dairy cows constitute an especially vulnerable group of food animals, and as such their welfare is of critical importance in fulfilling society’s stated obligations and moral responsibility to ensure that food animals do not suffer.

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